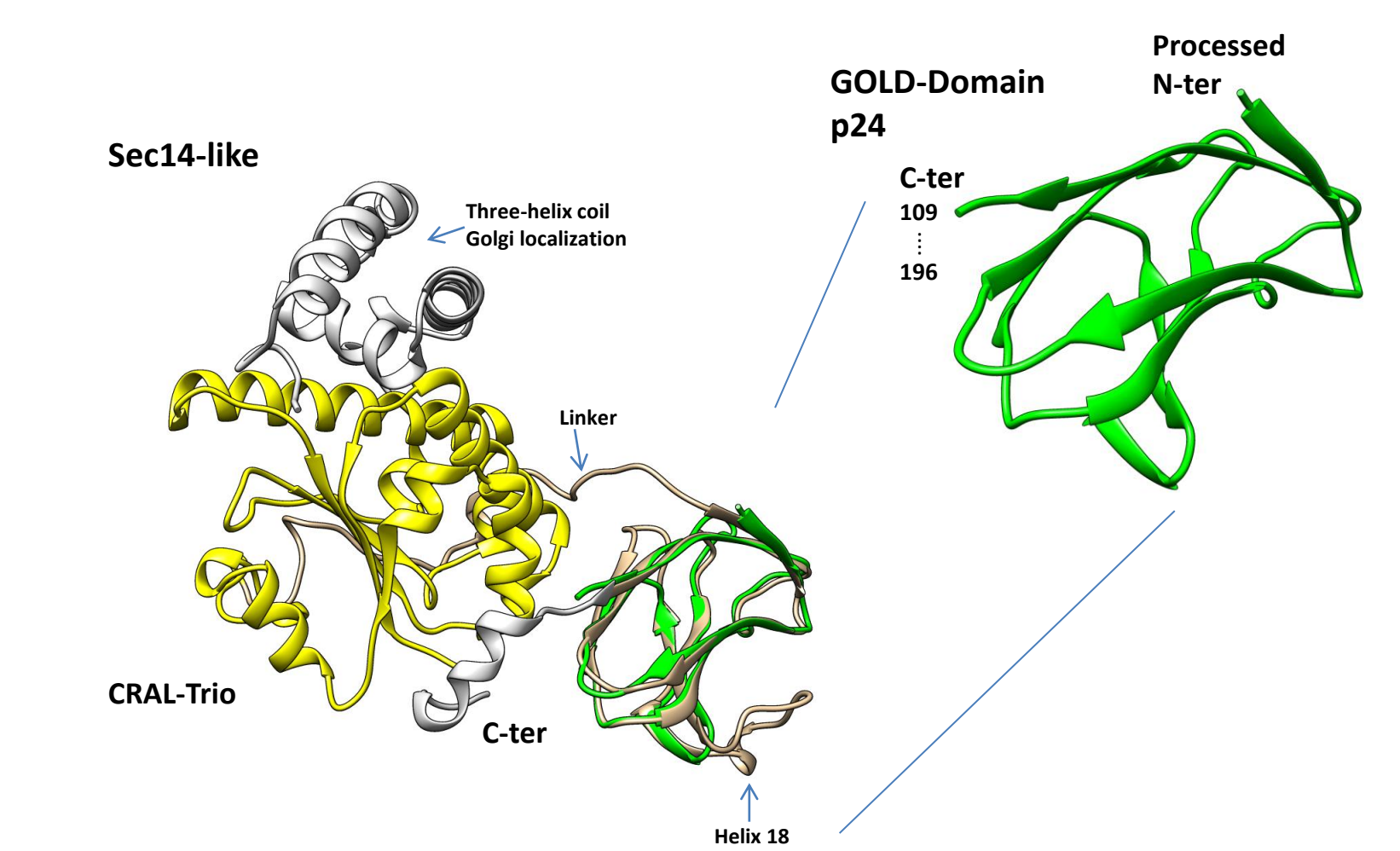


# p24-TMED Proteins Function as Cargo Receptors: The GOLD-Domain is Similar to a Collagen-Binding Domain and Carbohydrate-Binding Modules (CBMs)

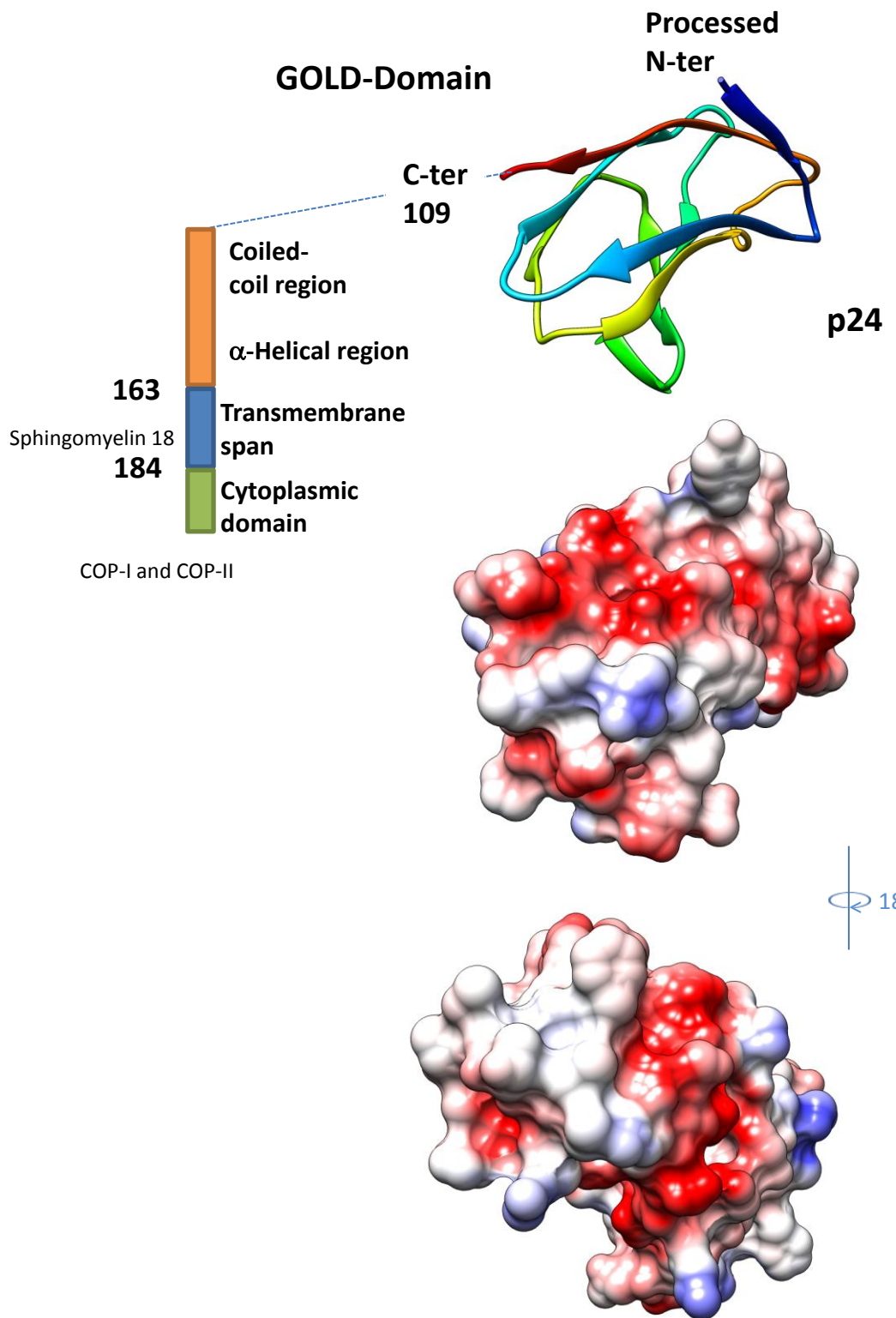
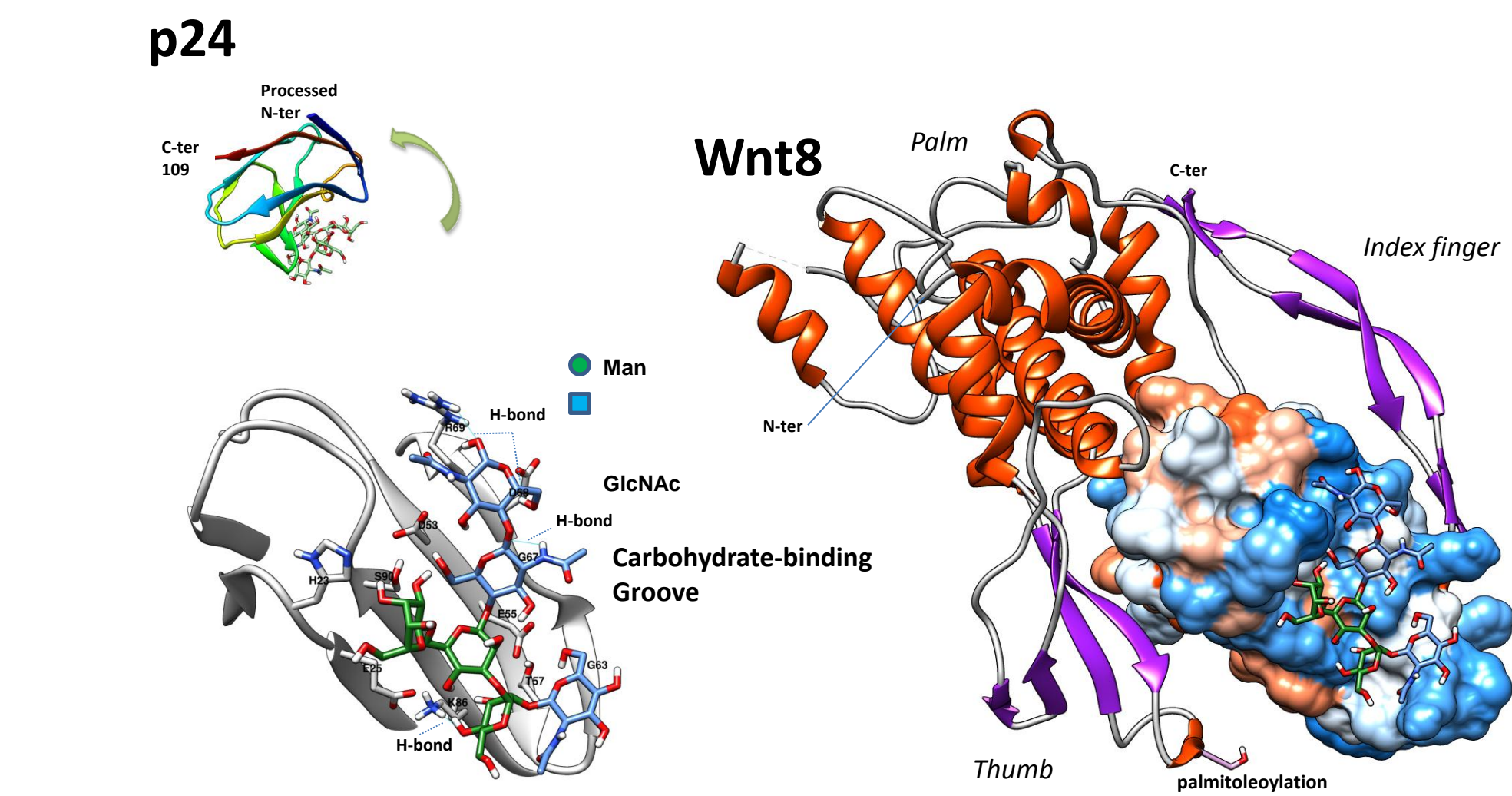
Klaus Fiedler

Apical transport in epithelia implicates lectins in recognition of N-glycans of glycoproteins. The Sec14-like protein (280-385 GOLD-domain) in this work scores highly with the carbohydrate-binding module (CBM) of the RetS domain from *Pseudomonas aeruginosa* and further CBMs. The modeled *Cricetulus griseus* p24-GOLD domain of p24, a member of intra-Golgi cargo receptors, is shown to interact with Wnt8 (wingless 8) of *Xenopus laevis* in a convincing ClusPro example of high score combined with a unique PDBePISA interface with a  $\Delta G=-18.3$  kcal/mol. Lower ranked models listed a smaller  $\Delta G$  (PDBePISA) and energy of DelPhi interaction. Complex/hybrid N-glycans provide increasing energy of binding up to -7.1 kcal/mol to simulated p24-GOLD-ligand interaction. It is likely, that Wnt proteins and p24 cargo-receptors interact analogously to Wnt-Fz.

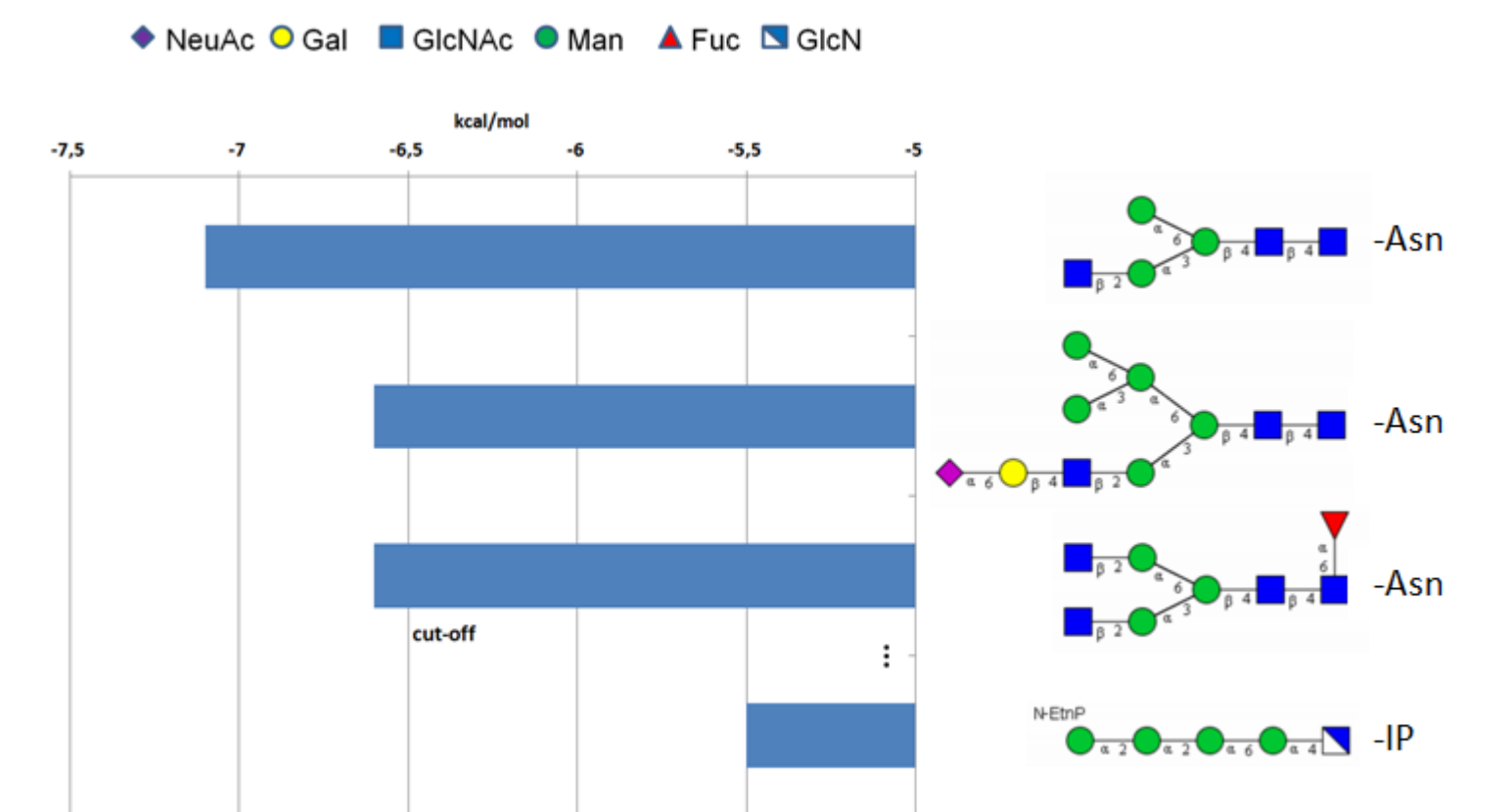


The Sec14-like protein and similarity to the p24-GOLD-domain. Sec14-like protein 3 includes two distinct domains, a CRAL-TRIO (yellow) and GOLD domain (gold). CRAL-TRIO binds to lipids (yeast Sec14) and tocopherol (human tocopherol transfer-protein); the orthologue Sec14-like protein 2 was crystallized and found to display a closed lipid-exchange loop (residue 196-215) which shields the hydrophobic binding cavity. The p24 GOLD-domain (green) models with crystallographic-like quality from residue 16 to 109. The eight-stranded fold of the GOLD domains is similar to unique bacterial collagen- and frequent carbohydrate-binding modules. A linker attaches the Sec14-like GOLD domains to the CRAL-TRIO (gold). Helix 18 (gold) of the Sec14-like protein 3 is indicated and shields one side of a concave face oriented opposite to the Golgi-localization three-helix coil (silver).

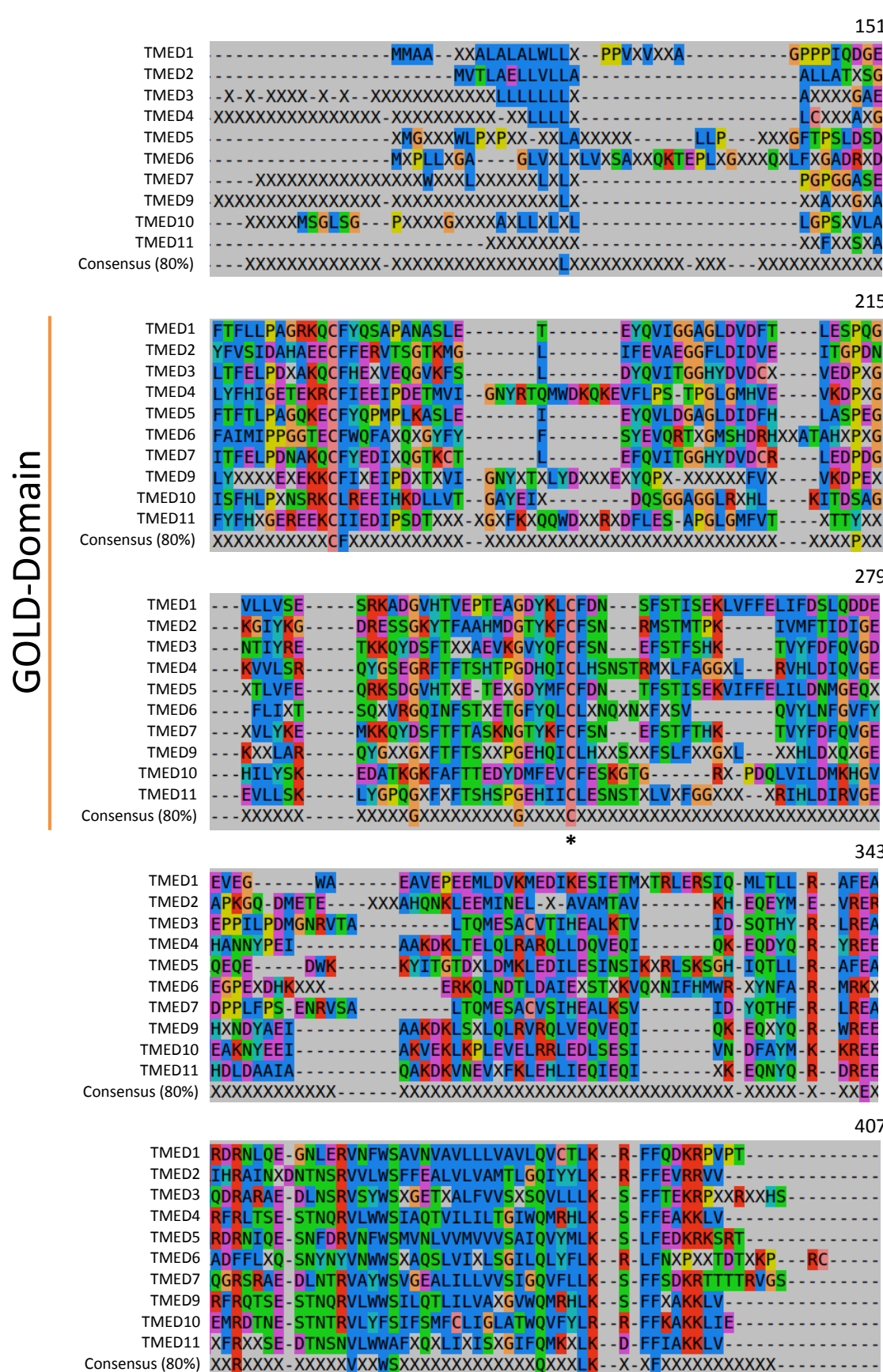
Modeled p24 GOLD-domain. The electrostatic surface presentation is shown in Coulombic coloring with identical (top panel) and rotated orientation.



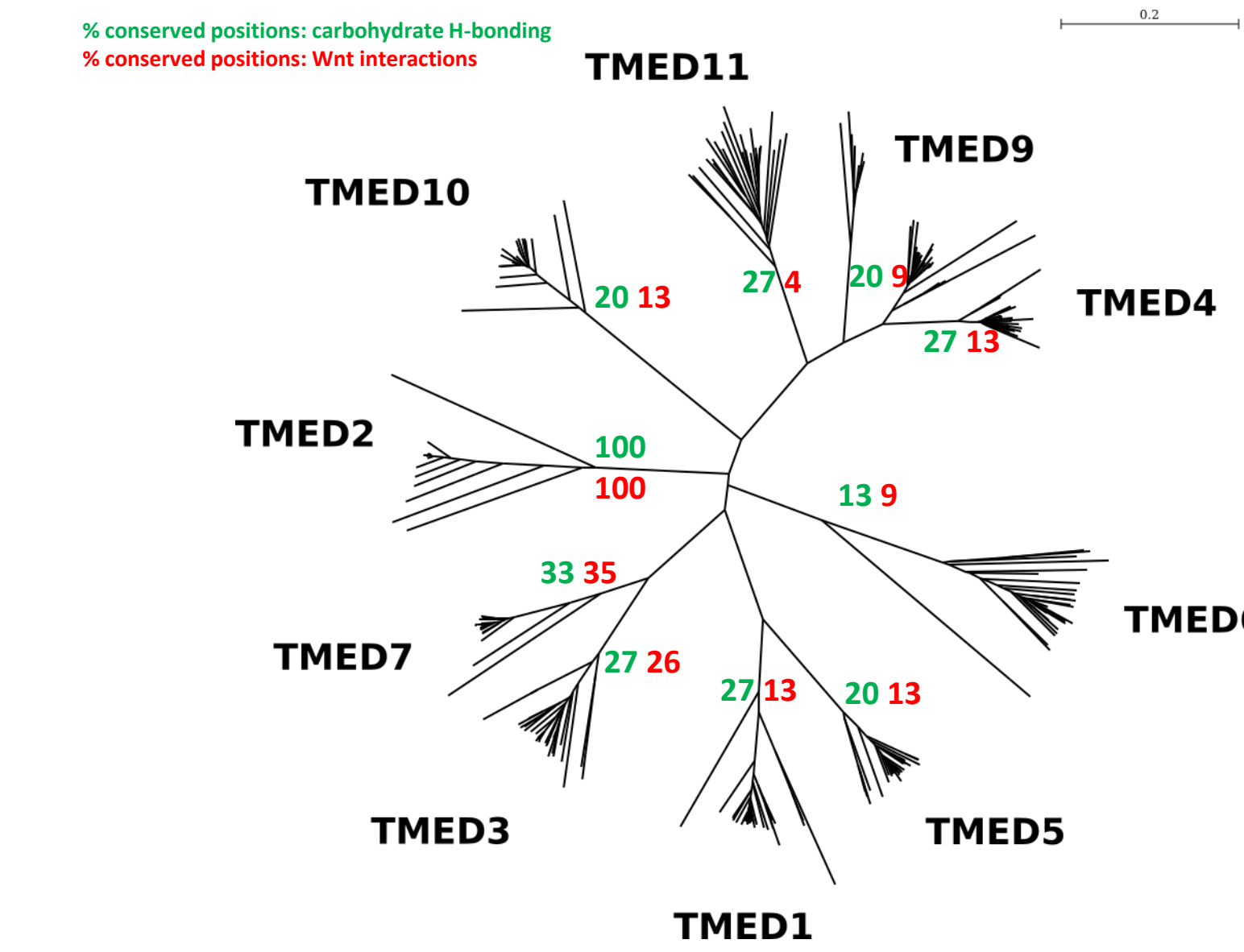
Docking of glycans. N-glycans of complex, hybrid and high-mannose type as well as some terminal glycan groups were docked to p24 (GOLD-domain). The HybridM3 glycan represented a top score, further hybrid and complex glycans scored lower (see right (IUPAC code)). The core of glycosylphosphatidylinositol-anchors was included for comparison. Asn- and phosphatidylinositol linkages (IP) are indicated.



Glycan docking and Wnt interaction. Wnt8 was found to interact with frizzled and biochemical interactions of p24 family members with Wnt proteins in *D. melanogaster* have been identified. Thus interaction of p24 cargo receptors with Wnt8 were studied in ClusPro-docking and identified a binding site of the GOLD-domain overlapping with the Fz8 cysteine-rich domain interface at the index finger and a thumb-binding site that was shifted towards the Wnt8 palm distant from the Wnt8 palmitoleoylation at Ser187. The palmitoleoyl group attaches to Fz8, stabilizes interaction and mediates Fz8 growth factor activity. Although thumb binding is not identical the p24 family proteins may act as a decoy in Wnt interaction and as cargo-receptors. A carbohydrate binding groove was identified in p24 from CHO with intermediate affinity (-7.1 kcal/mol) which is similar to other lectins with affinity of 10<sup>4</sup> M<sup>-1</sup> to N-glycans [1]. The hybrid glycan (GlcNAc-core-Man3-GlcNAc) is colored in IUPAC code (left panels). Putative carbohydrate binding at Glu55, Thr57, Gly63 and Lys86 (p24) would be inhibited in equivalent positions in Sec14-like proteins with closed Helix 18 conformations. Residues on the concave face of p24 are labeled within a distance of relaxed hydrogen-bonding. H-bonds [49] are indicated. Wnt8 is shown in secondary structure coloring (α-helix, red; sheet, magenta), the p24 surface is colored by hydrophobicity (red, hydrophobic; blue, hydrophilic).



Clustal multiple-sequence alignment of mammalian (ENSEMBL) TMED sequences. 290 sequences were selected that did not contain gaps within the GOLD-domain (due to inaccurate sequence reads or splicing). The 80 % consensus of each TMED protein and the family consensus is presented. TMED4, 9, 10 and 11 contain inserts within the GOLD-domain (indicated). X represents the no-consensus position. Few amino acids are preserved in the whole family at the 80 % level, these accumulate at the transmembrane junction and within the transmembrane span, two, the Q and F (indicated with an asterisk) have been described as traffic signals [2, 3], the C within the GOLD-domain may be implicated in fold generation and/or collagen/carbohydrate binding.



Phylogenetic tree of TMED proteins. All mammalian sequences were collected from ENSEMBL and inaccurate sequence reads were deleted - 326 sequences were aligned by Clustal and presented in Newick format, actual distances are indicated in the graphical representation. TMED7 is close to TMED2 and comparison of 15 residues involved in hydrogen bonding to carbohydrate (or in close proximity) in TMED2 shows that 33 % (green) of these are conserved/conservatively replaced in TMED7, out of 23 residues implicated in Wnt binding 35 % (red) are conserved in TMED7 (identity), corresponding numbers are indicated for the other proteins; other sequences show a higher substitution rate.

Interactions from database (including some genetic interactions)

1. Fiedler K (2014-) Apical transport in epithelia. *submitted*
2. Fiedler K, Veit M, Stamnes MA & Rothman JE (1996) Bimodal interaction of coatomer with the p24 family of putative cargo receptors. *Science* 273: 1396–1399
3. Goldberg J (2000) Decoding of sorting signals by coatomer through a GTPase switch in the COPI coat complex. *Cell* 100: 671–679