Structural basis of the interaction between integrin α6β4 and the bullous pemphigoid antigen BP230 in hemidesmosomes

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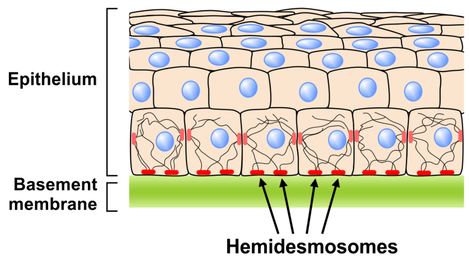
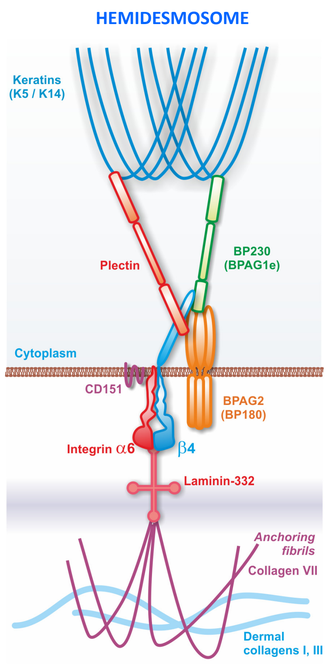
- **BP230 and plectin link integrin α6β4 to keratin filaments in hemidesmosomes.**
- **Crystal structures of β4-BP230 complex solved.**
- **BP230 induces a conformational change of in β4 as observed by DEER.**
- **Phosphomimetic mutations in BP230 inhibit binding to β4.**
- **Binding of BP230 to β4 is necessary for recruitment of BP230 into hemidesmosomes.**



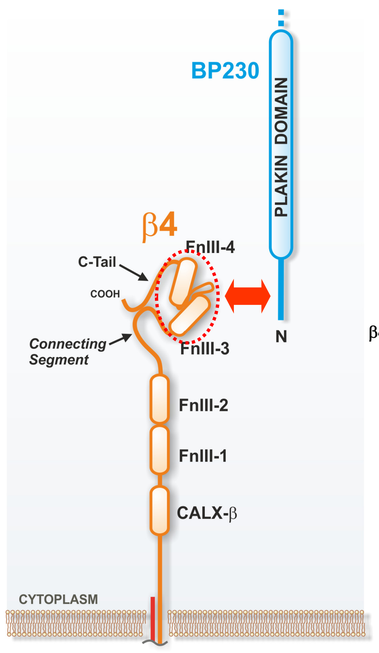
HEMIDESMOSOMES: components and interactions



Hemidesmosomes (**HDs**) are complexes that mediate the stable adhesion of epithelial cells to the basement membrane by linking the extracellular matrix to the intermediate filament system of the cytoskeleton.

In (pseudo-)stratified epithelia HDs contain **integrin α6β4**, the bullous pemphigoid antigen 2 (**BPAG2**, also known as BP180), the tetraspanin **CD151**, **plectin**, and **BP230** (BPAG1e).

Integrin α6β4 is connected to the cytokeratins by plectin and BP230, two proteins of the plakin family of cytolinkers.



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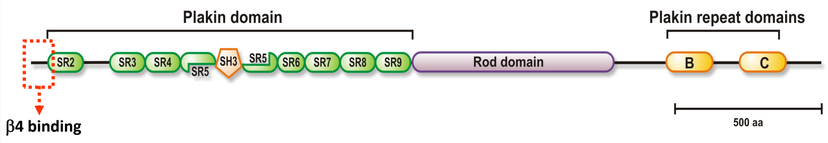
BP230 has a tripartite structure: the N-terminal region is occupied by a short **N-terminal tail** and a large **plakin domain**, the central segment contains a coiled-coil **rod domain**, and the C-terminal region contains two **plakin repeat domains**.



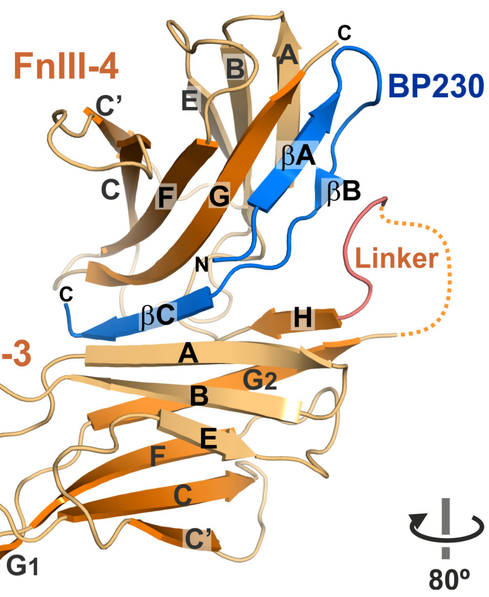
The **integrin β4** subunit has a uniquely large **cytoplasmic tail** that contains four **fibronectin type III domains (FnIII-1 to FnIII-4)**.

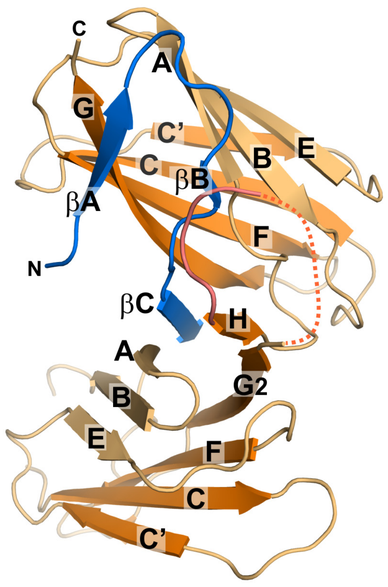
Here, we have mapped the mutual binding sites in integrin β4 and BP230. We have characterized the structural basis of the interaction and we show that it is required for the assembly of complete HDs in human keratinocytes.

Crystal structure of the BP230-integrin β4 complex

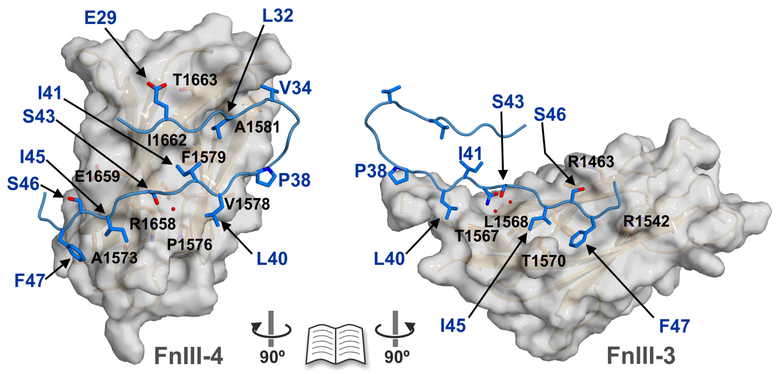
Binding occurs between a short segment of the N-terminal tail of BP230 (residues 26-55) and the region FnIII-3,4 of integrin β4.
We have elucidated two crystal structures of the **FnIII-3,4 of β4** bound to the **N-terminal region of BP230 (26-55)**:

Structure	PDB entry	Resolution
β4 **FnIII-3,4 (WT) + BP230**	6GVL	2.05 Å
β4 **FnIII-3,4 (T1663R) + BP230 High affinity mutant**	6GVK	1.55 Å

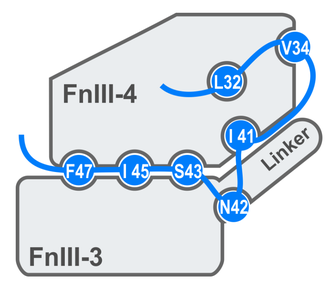




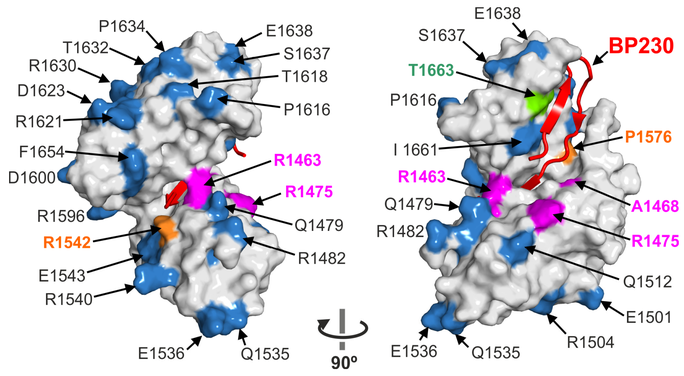
Conserved residues in BP230 insert into pockets of β4-FnIII-3,4
Open-book view of the complex: surface representation of the FnIII-3 (left) and FnIII-4 (right) and wire representation of BP230 showing residues docked into β4.



Representation of the interactions of BP230 residues with β4.



We had combined mutagenesis with quantitative analysis of the interaction to map the BP230-binding site in β4.
The **mutagenesis data** are in **agreement** with the structure of the complex and reveal residues that mediate key interactions.



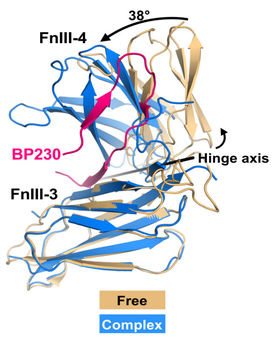
Affinity of integrin β4 mutants for BP230, Kd (μM)

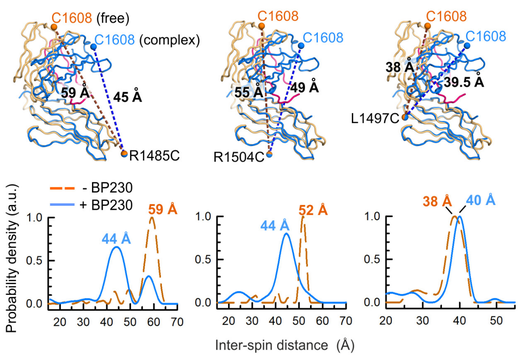
<40	
40-100	Wild type
100-200	
200-800	
>800	

BP230 induces a closure of the FnIII-3,4 of β4

Superimposition of the structures of the FnIII-3,4 in the **free form** (orange) and **bound to BP230** (β4 blue and BP230 magenta).

We have characterized the conformational change using double electron-electron resonance (**DEER**).

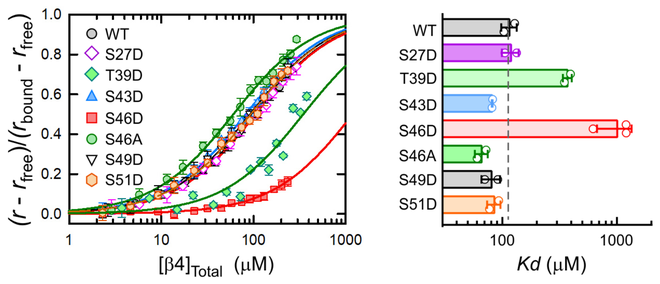


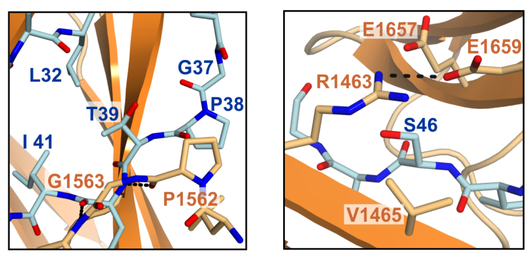


Phosphomimetic mutations in BP230 inhibit binding to β4

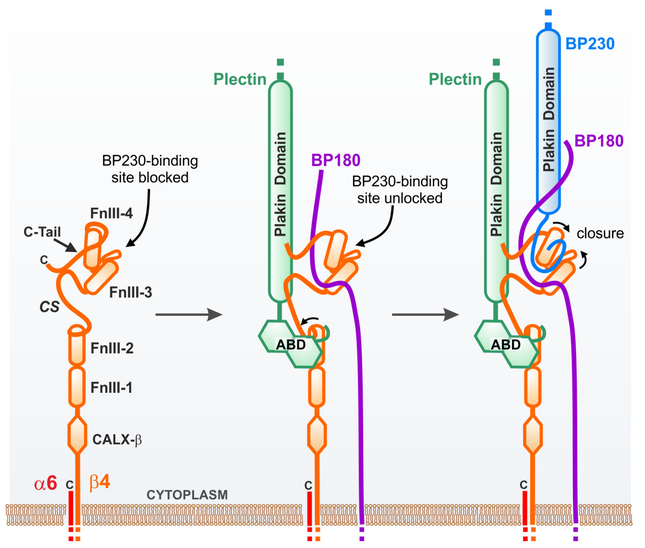
Phosphorylation of β4 at Ser residues disrupts the interaction with plectin and it is linked to hemidesmosome disassembly. This prompted us to examine the role of Ser residues in the BP230-β4 interaction.

The β4-binding site in BP230 contains several Ser. The substitutions **T39D** and **S46D** in BP230 reduce binding to β4. Suggesting that the BP230-β4 interaction could be regulated by phosphorylation.





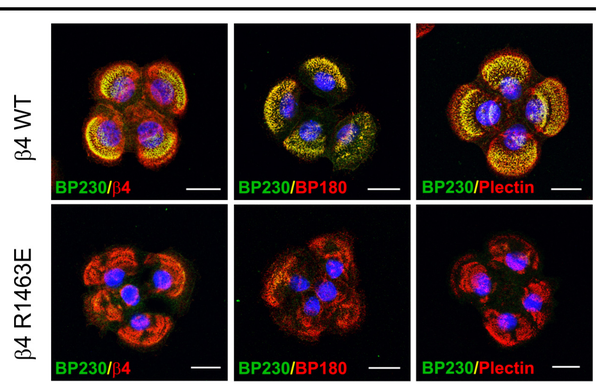
Model of regulation of BP230-β4 interaction during hemidesmosome assembly



Binding to β4 is necessary for recruitment of BP230 into hemidesmosomes

Distribution of BP230, plectin, and BP180 was analyzed in human PA-JEB **keratinocytes** derived from a patient in which WT or mutant β4 was expressed (PA-JEB keratinocytes do not express endogenous integrin β4).

Mutation **R1463E** blocks binding to BP230 *in vitro* and prevents recruitment of BP230 into hemidesmosomes in cells.



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