Uncovering the association mechanism between two intrinsically flexible proteins

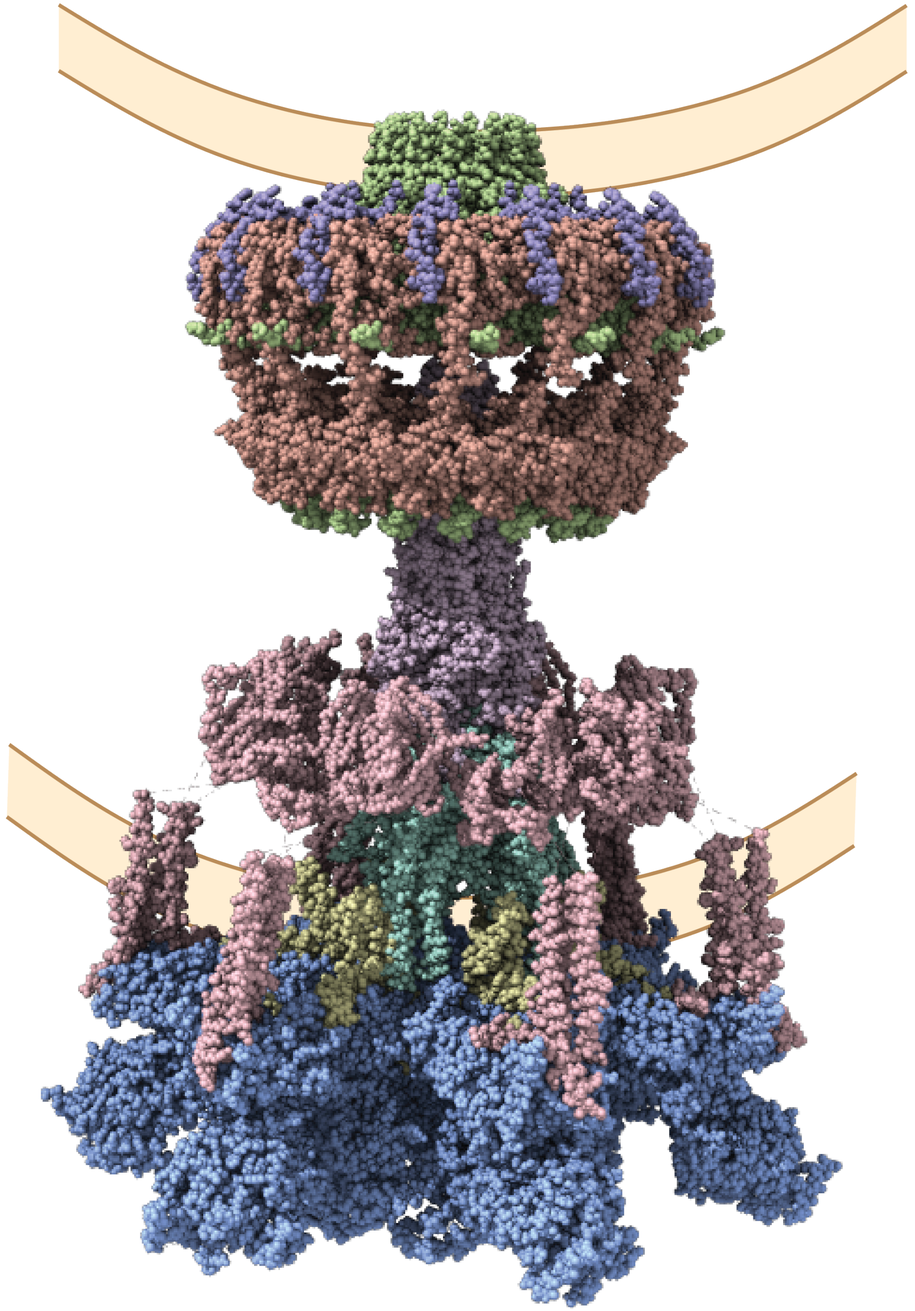
Roberto K. Salinas, IQUSP

- **Bacteria use a variety of Secretion Systems to transport macromolecules across the cellular envelope**

- **Among them, the T4SS translocates DNA (conjugation systems) or proteins (effector translators)**

- **T4SS are nanomachines with 1 - 3 MDa formed by 11 VirB subunits (VirB1 - VirB11)**

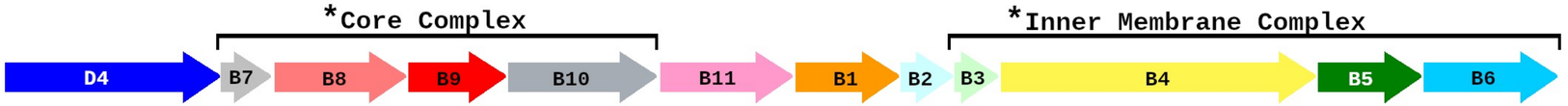
- **Involved in the spread of antibiotic resistance and infection by pathogens**



Mace et al. (2022) *Nature* **607**: 191 (PDB: 7O41, 7O3V, 7O3T, 7O3J)

The bacteria *Xanthomonas citri* contains one T4SS locus in the chromosome

Xanthomonas citri cromossomal *virB* locus (T4SS)



Xanthomonas citri VirB7 is larger than the canonical VirB7

```
Xac B7       22     CATKPAPDFGGRWKHVNHFDEAPTEIPLYTSYTYQATPMDGTLKTMLERWAADSNMQLSYNLPSDYTLIG
E.coli B7    15 CSSGHKPPPEPDWSNT-VPVNKTIPVDTQGGRNES-------------------------------------

Xac B7       92 PVSAISTTSVQQAATELSAVYAAQGVSVSVSANKLLVQPVPVSSGAKL 139
E.coli B7       ---------------------------------------------
```

The $T4SS_{XAC}$ secretes toxins that kill other gram-negative bacteria on a contact-dependent basis

Diorge Souza et al. (2015) *Nature Commun.* **6**: 6453

Xanthomonas citri VirB7 is larger than the canonical VirB7

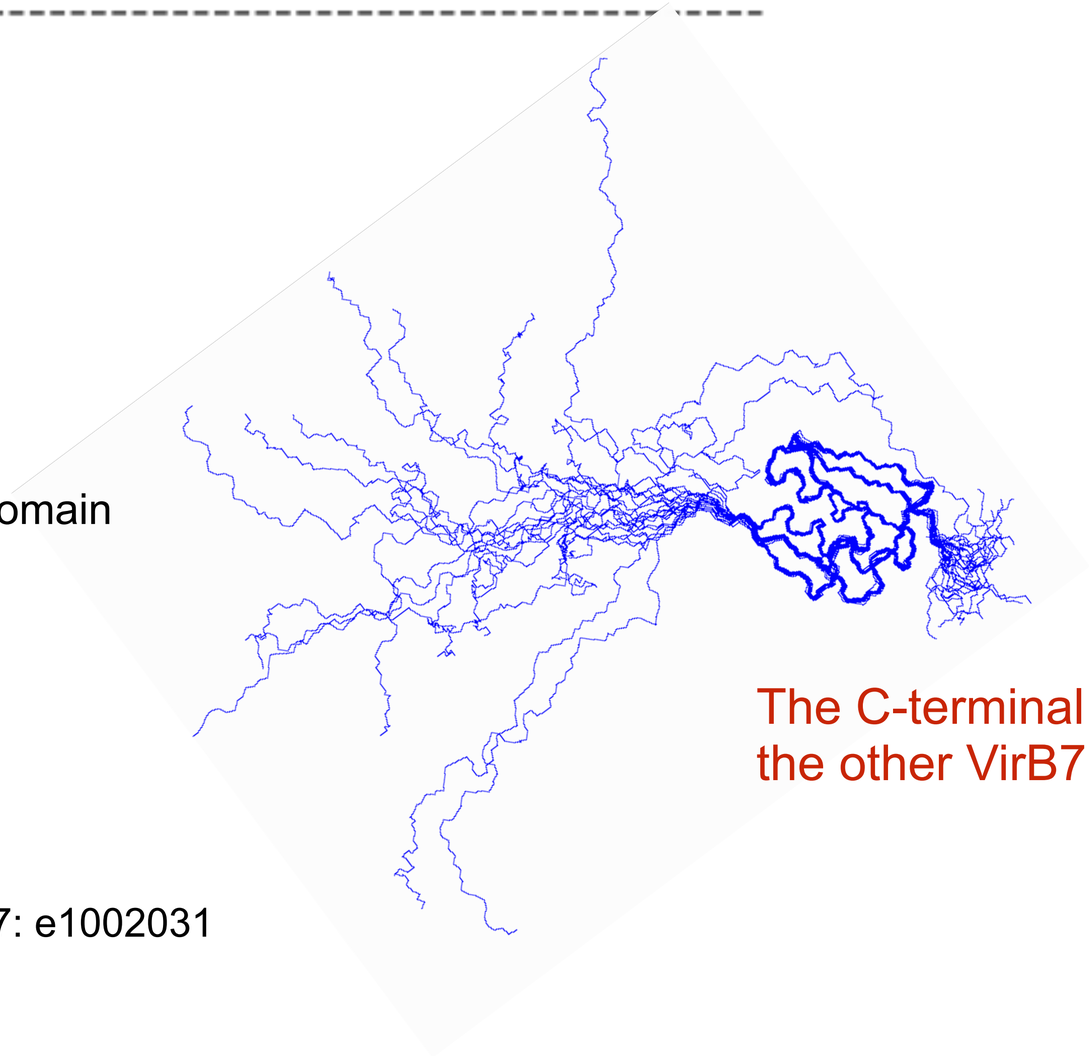
```
Xac B7        22     CATKPAPDFGGRWKHVNHFDEAPTEIPLYTSYTYQATPMDGTLKTMLERWAADSNMQLSYNLPSDYTLIG
E.coli B7     15 CSSGHKPPPEPDWSNT-VPVNKTIPVDTQGGRNES-------------------------------------

Xac B7        92 PVSAISTTSVQQAATELSAVYAAQGVSVSVSANKLLVQPVPVSSGAKL 139
E.coli B7        ---------------------------------------------
```

Canonical VirB7

N-terminal IDR

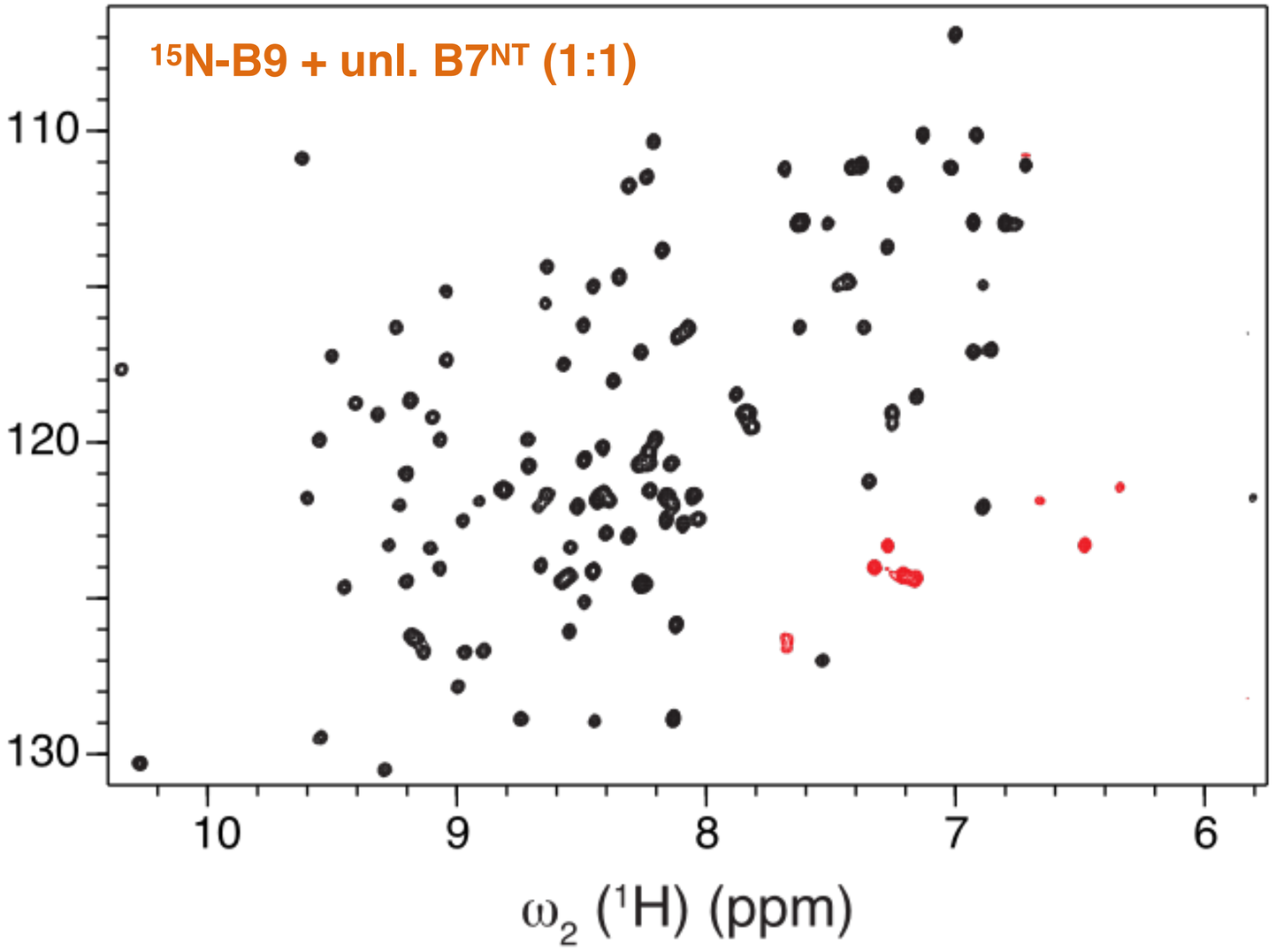
C-Terminal Domain



The C-terminal domain is absent in the other VirB7 proteins

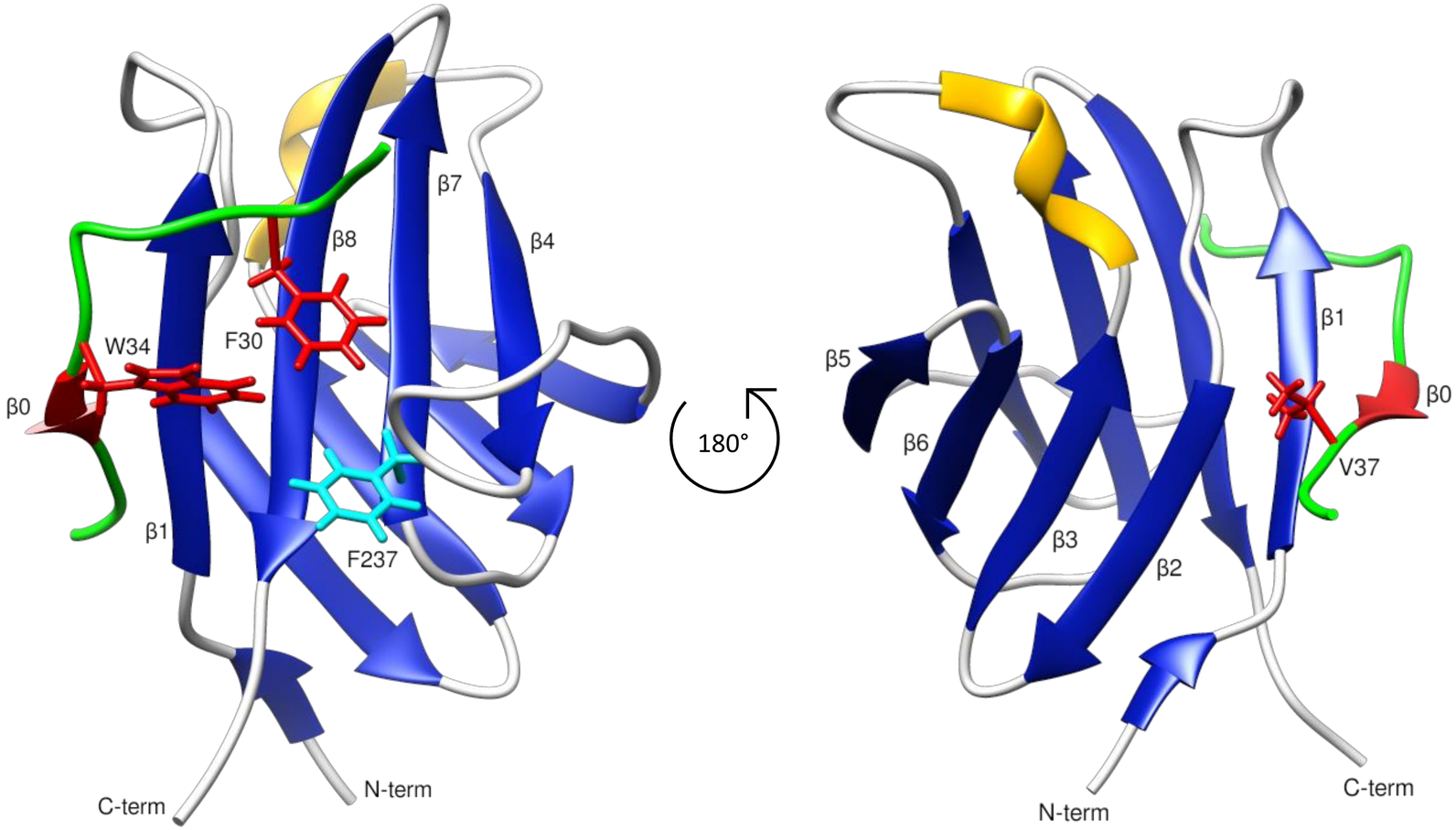
Souza *et al*. (2011) PLoS Pathogens 7: e1002031

The disordered VirB7 N-terminal region binds to the VirB9 C-terminal domain forming a rigid complex



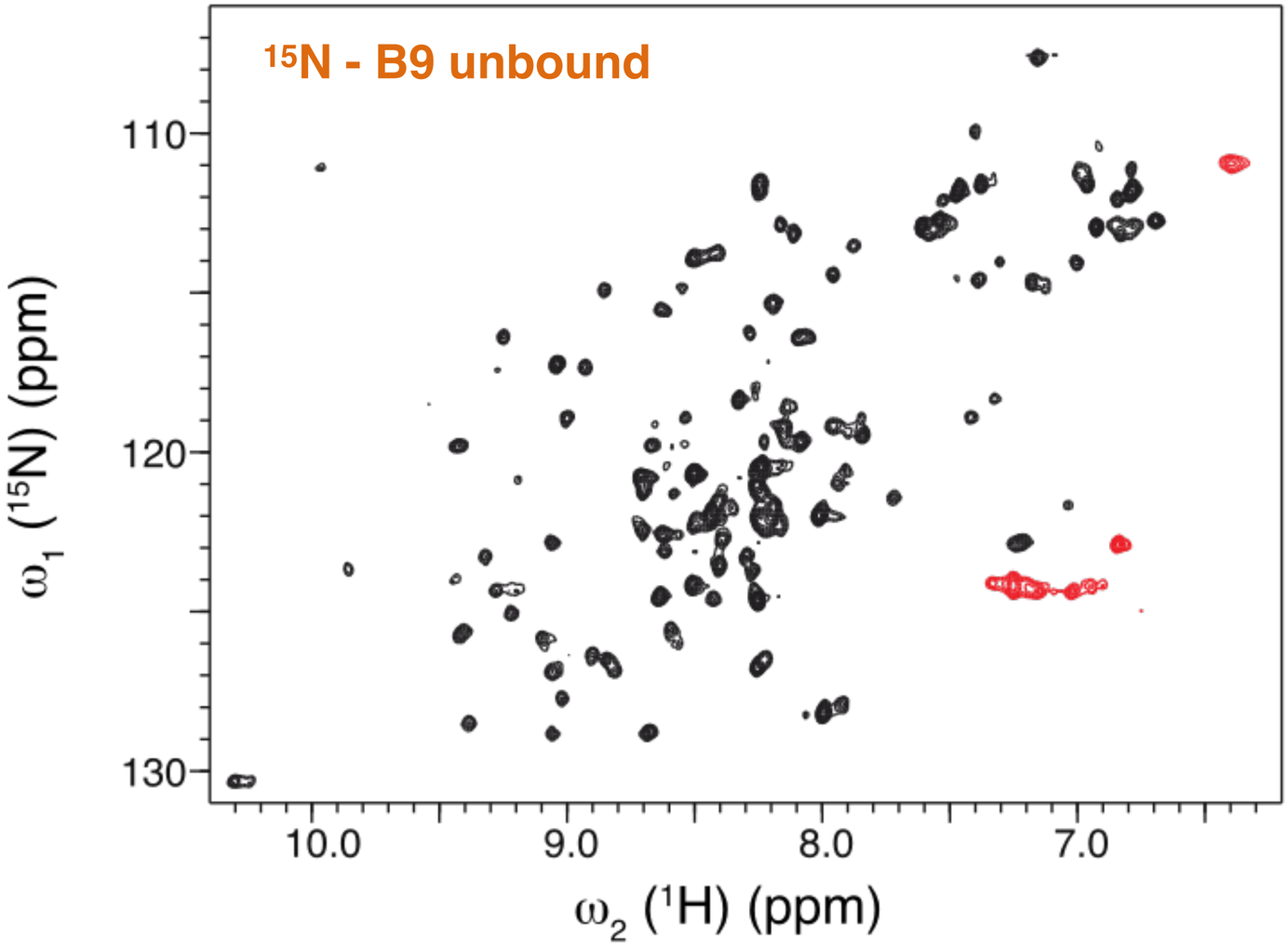
Oliveira *et al*. (2016) Structure

VirB7 N-terminal tail folds into a short ß-strand upon binding to the VirB9 C-terminal domain



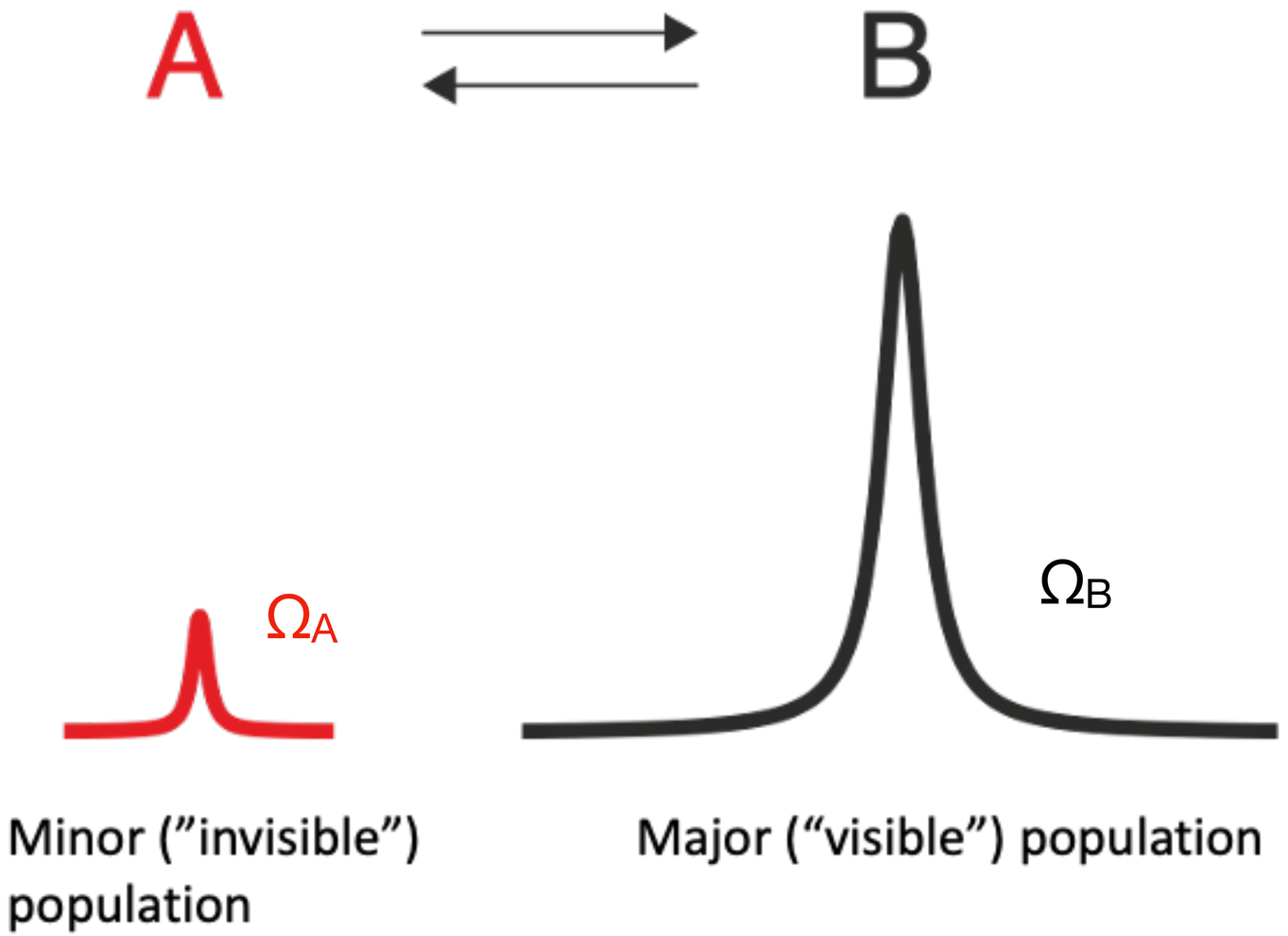
Oliveira *et al*. (2016) Structure

Qualitative analysis of the VirB9 C-terminal domain HSQC spectrum indicates that this domain is highly flexible in the unbound state

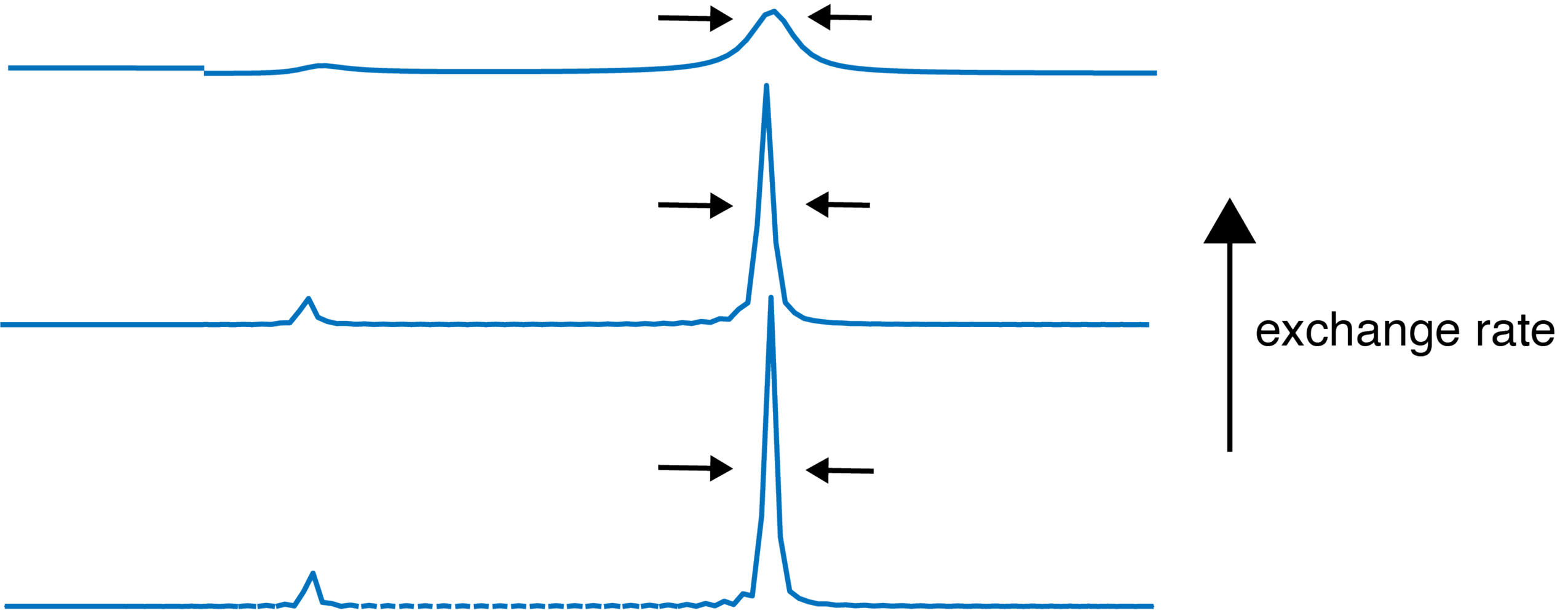


Oliveira *et al*. (2016) Structure

Conformational and chemical exchange processes (ex. association and dissociation) may significantly affect the NMR line shapes



NMR peaks may become broader or sharper depending on the exchange rate



The linewidth of NMR signals may contain information on dynamic processes that occur at the chemical shift time scale

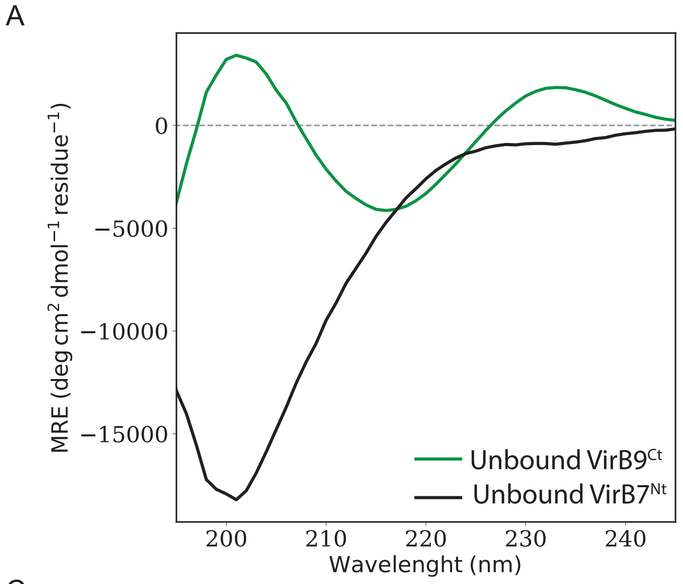
How does VirB7 and VirB9 recognize each other despite being highly flexible?

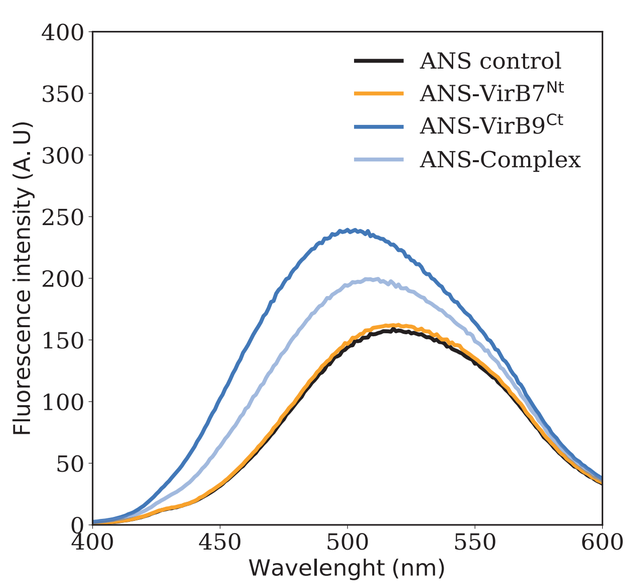
- Angie Dàvalos (IQUSP)
- Dr. José David Rivera Echeverri (IQUSP)
- Denize C. Favaro (IQUSP)
- Iolanda Cuccovia (IQUSP)
- Chuck Farah (IQUSP)
- Ronaldo Junio (UFTM)

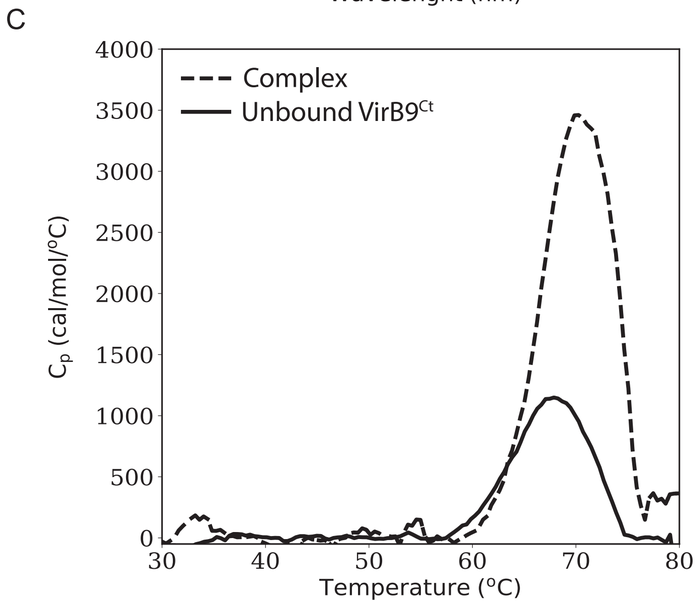
Davalos et al. (2024) ACS Chemical Biology

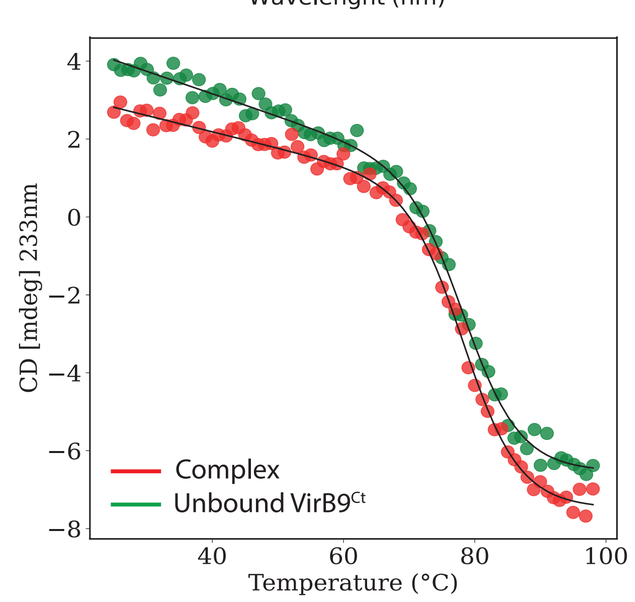
VirB7^{NT} CD spectrum is typical of a random coil peptide

The number of VirB9^{CT} residues in β-type conformation at the bound and unbound states differ by 7

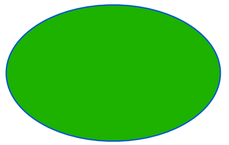








Canonical VirB7
VirB7^{NT}



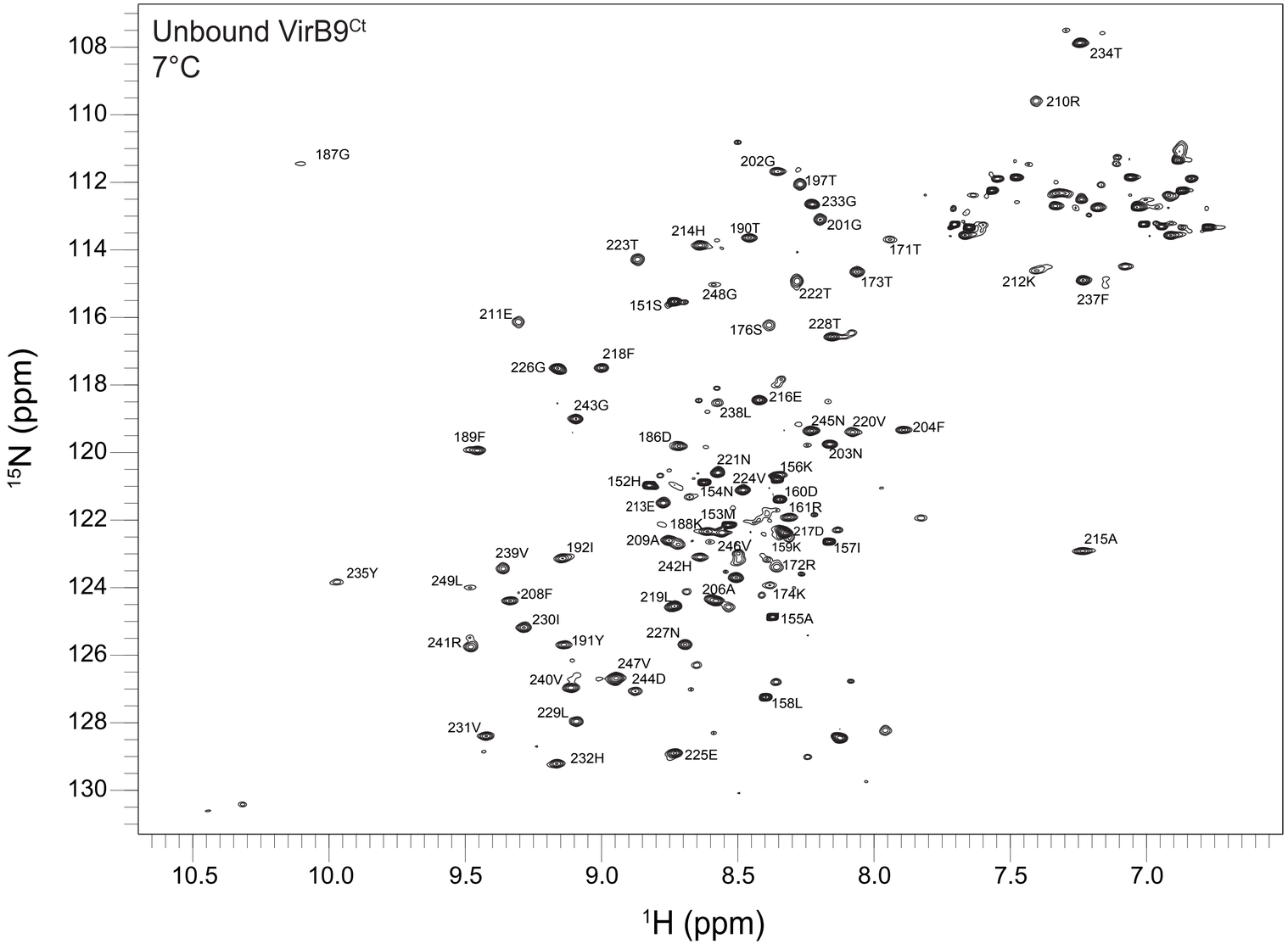
VirB9 C-Terminal Domain

CD spectral deconvolution

Secondary structure	Unbound VirB9^{CT} (%)	Unbound VirB7^{NT} (%)
α-helix	0	0.04
β-type	37	21
turns	26	16
rc	36	58

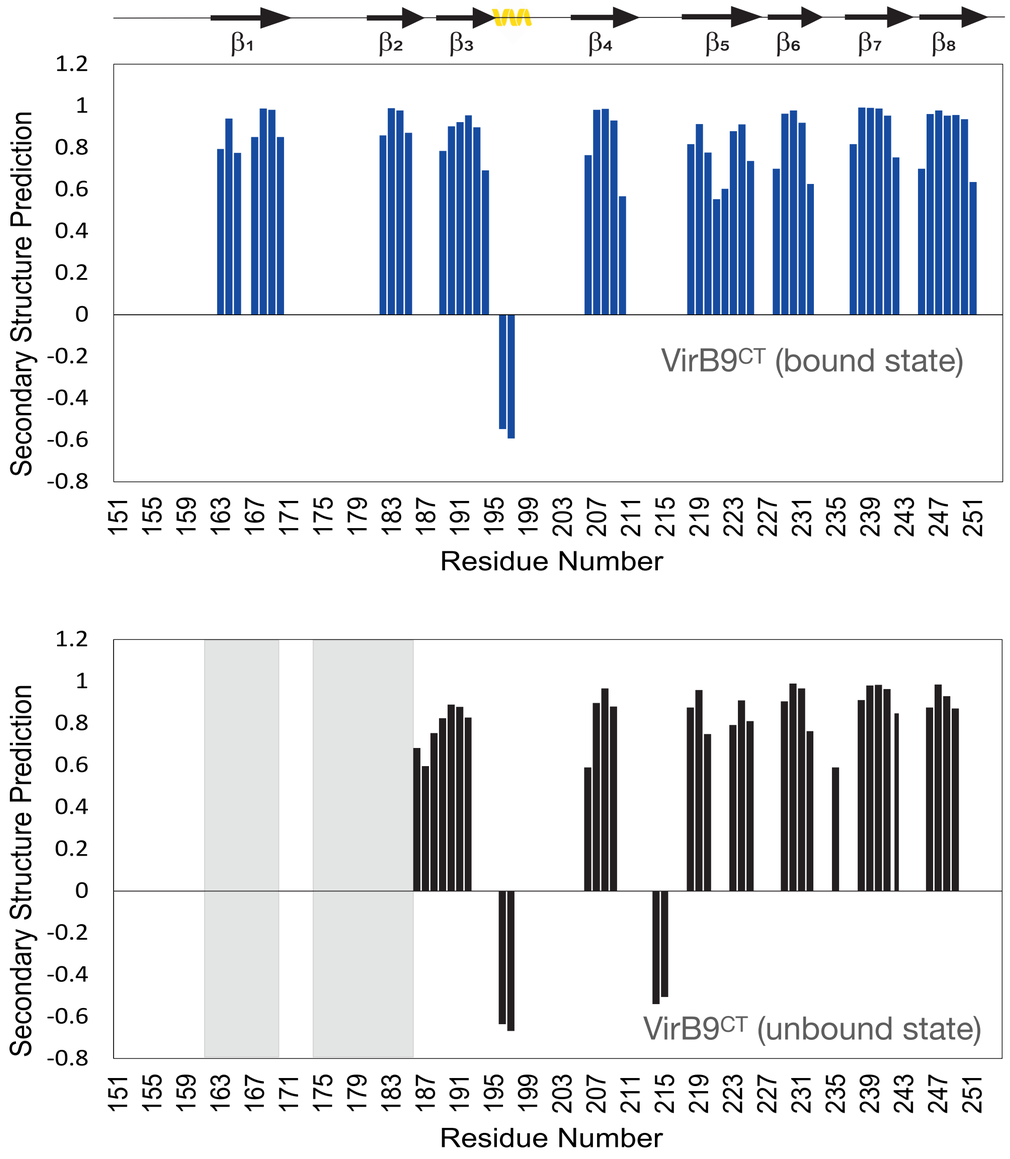
The number of VirB9^{CT} residues in β-type conformation in the bound and unbound states differ by 7

VirB9^{CT} dynamics becomes more restricted at low temperatures

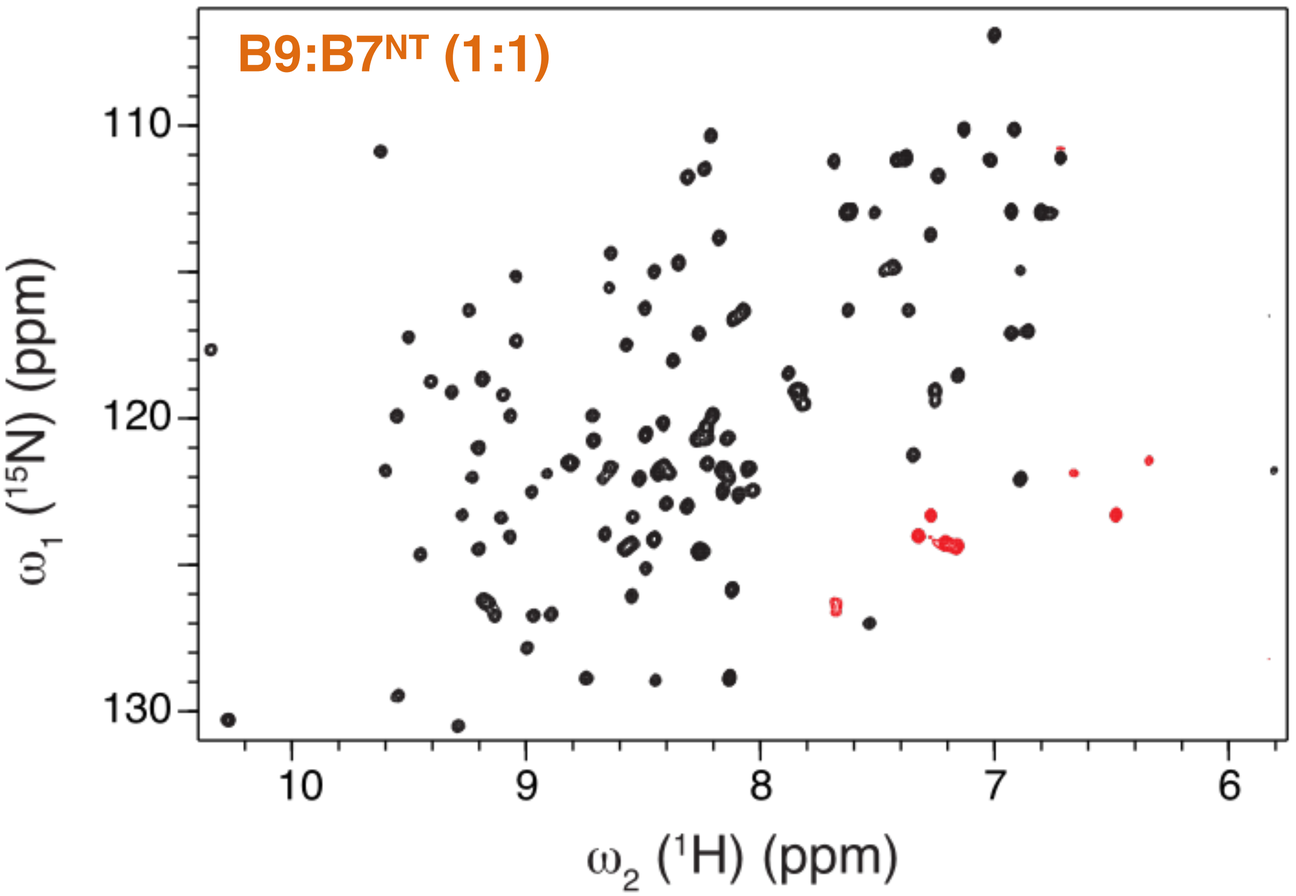


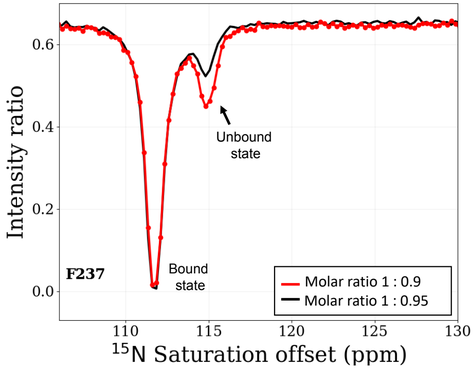
Increased spectral quality at 7 ˚C allowed us to obtain backbone resonance assignments for VirB9^{CT} in the unbound state

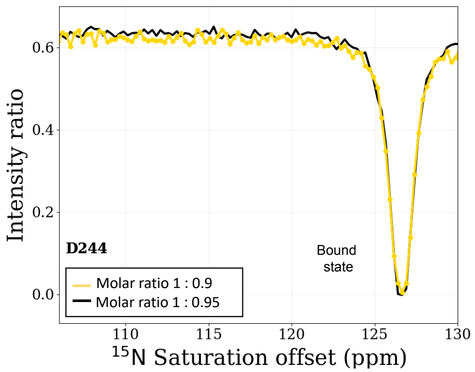
Analysis of NMR chemical shifts using TALOS indicated that most of the VirB9 ß-strands were already formed in the unbound state, with the exception of ß1 and ß2 that could not be assigned

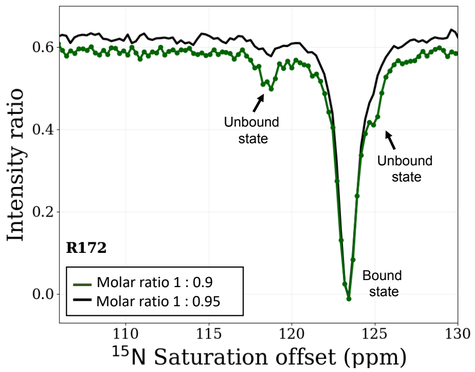


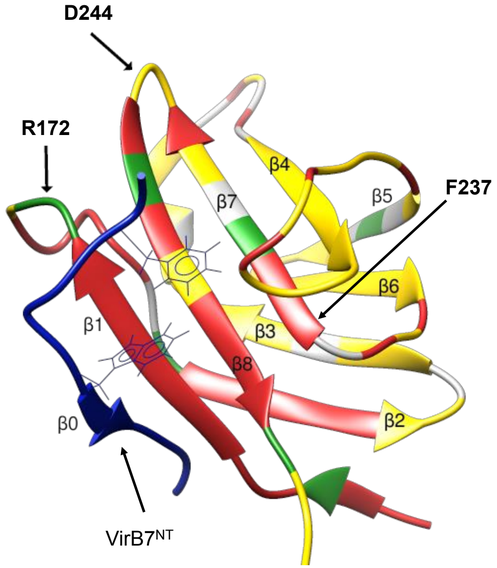
^{15}N chemical shifts at the invisible β1 and β2 (in the unbound state) were obtained using CEST (at 35 ℃)

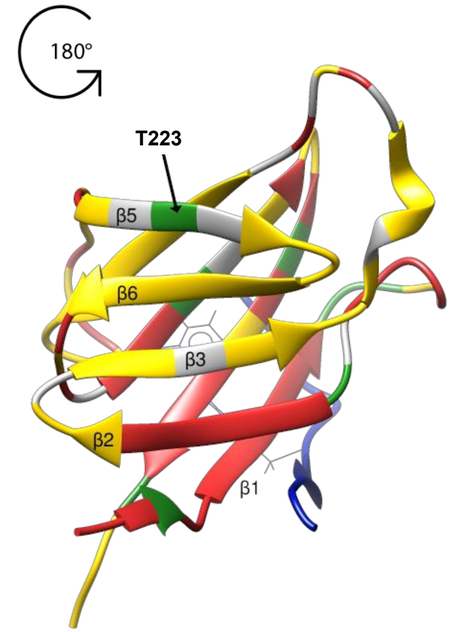




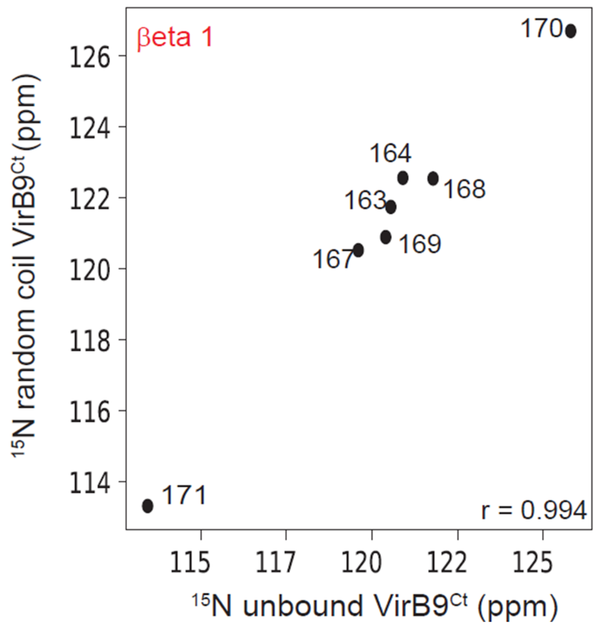




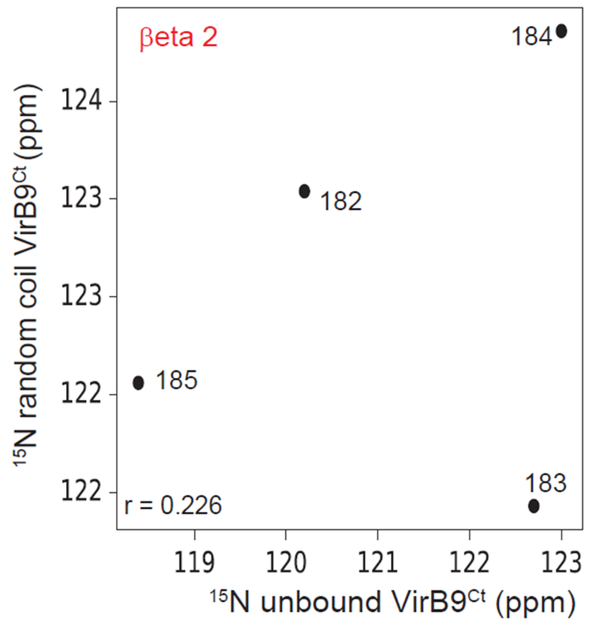




β1 seems to be disordered while β2 is folded in the unbound state



B



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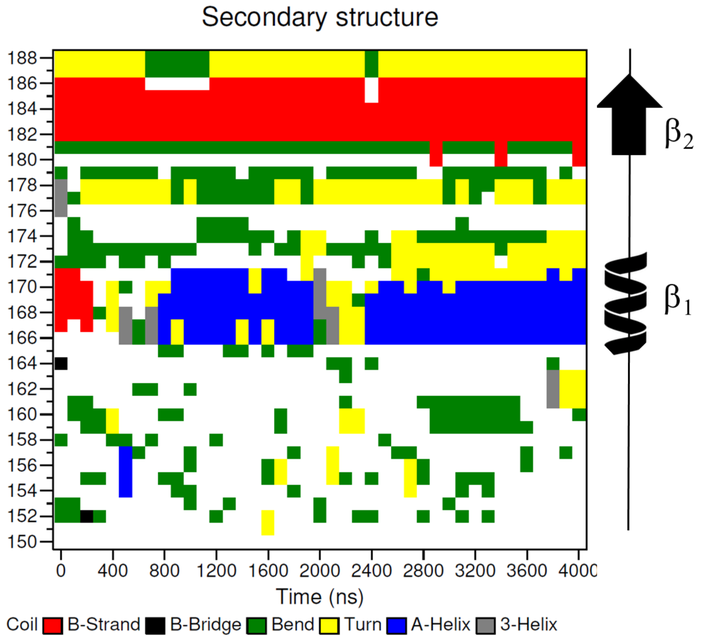
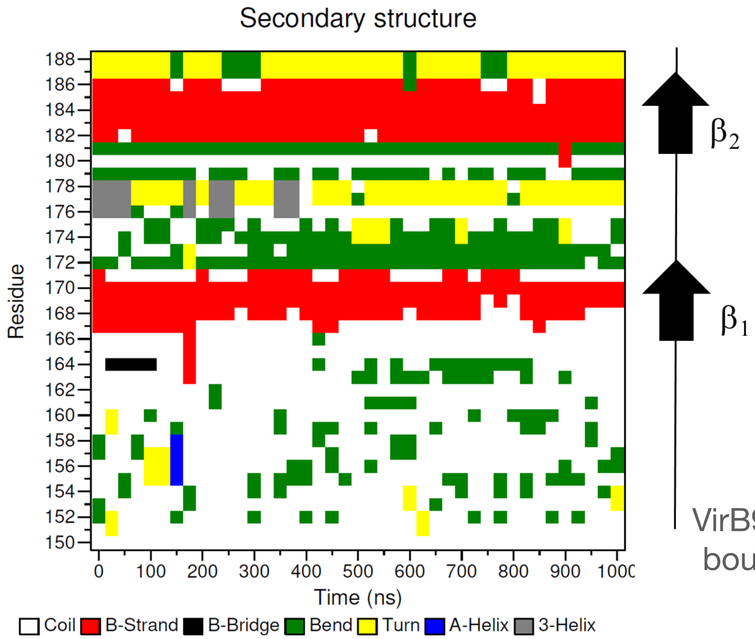


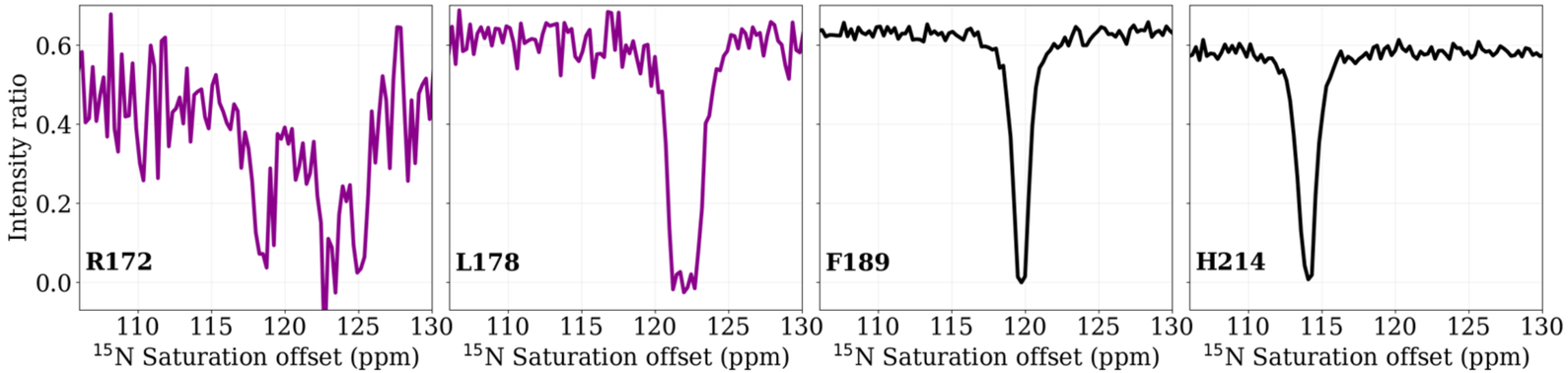
VirB9CT in the unbound state



VirB9CT in the bound state

The observation of multiple unbound state δ_{15N} leads to the question of whether VirB7^{NT} binds a “bound-like” VirB9^{CT} conformation?

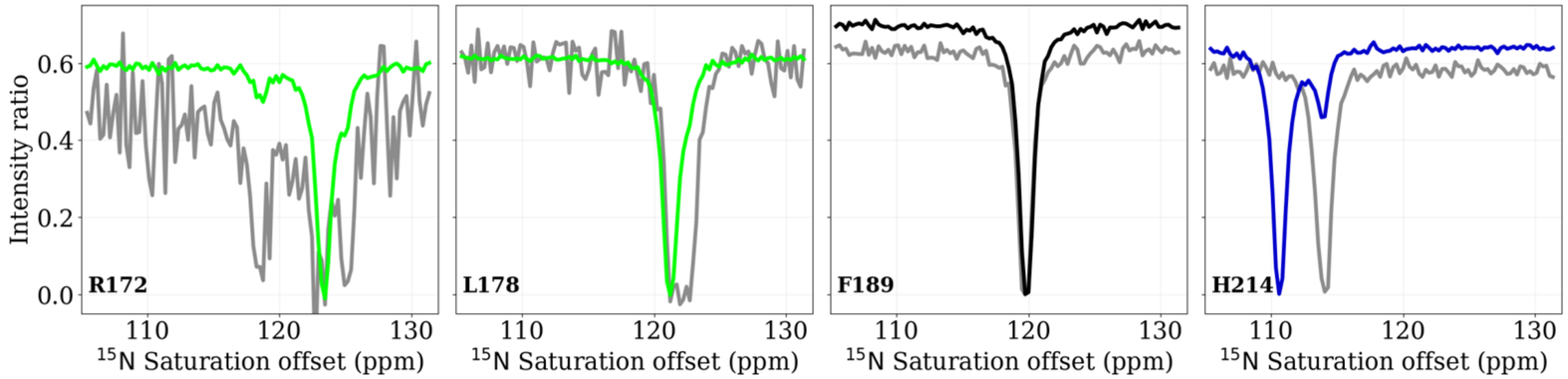
^{15}N-VirB9^{CT} alone



^{15}N-VirB9^{CT} alone

versus

^{15}N-VirB9^{CT} + VirB7^{NT}



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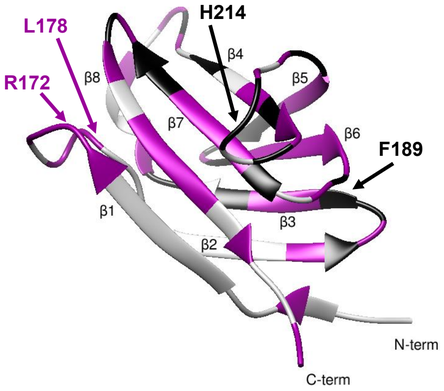
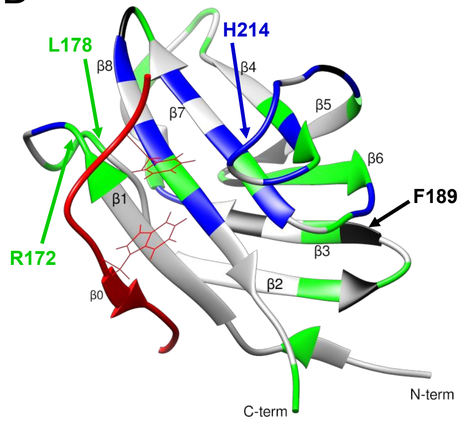
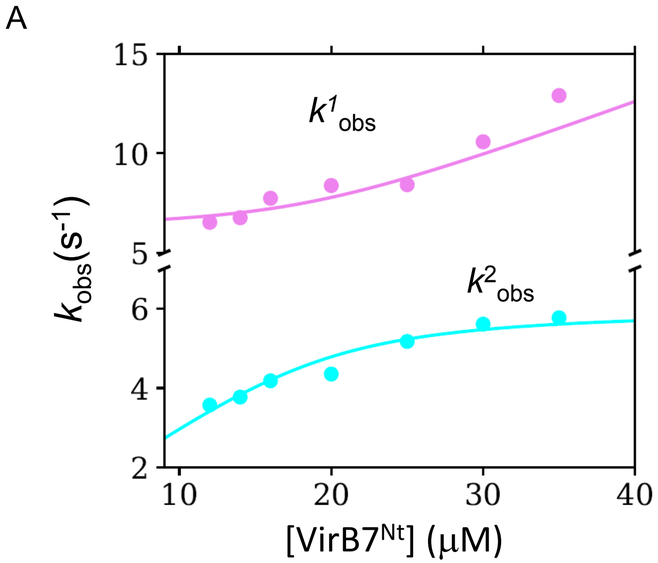


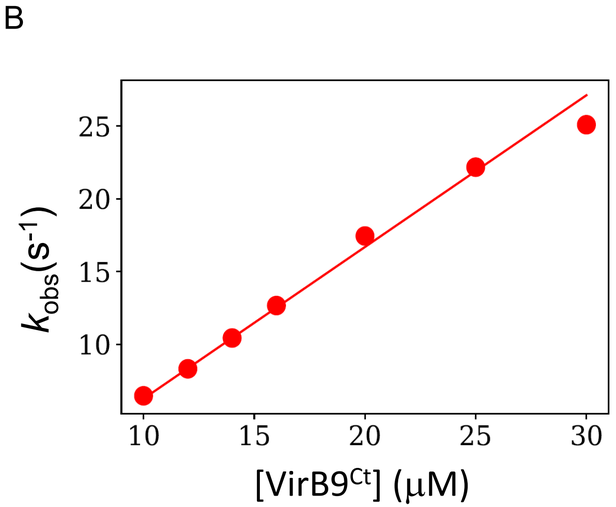
D



Protein association kinetics was followed by fluorescence stopped flow at 25 and 35 ℃ under excess of VirB7^{NT} (left) or VirB9^{CT} (right)

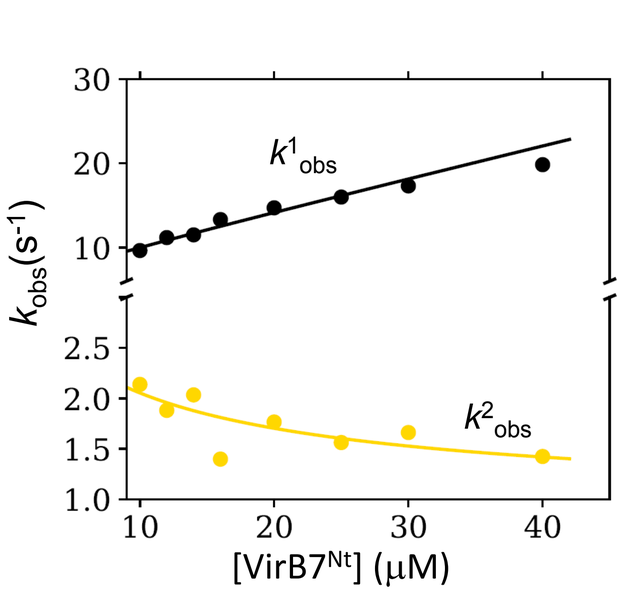
25 ℃

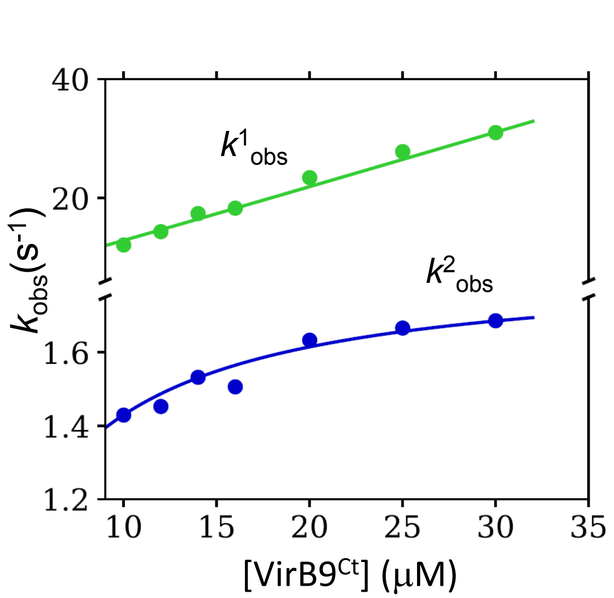




Binding follows a conformational-selection mechanism

35 ℃

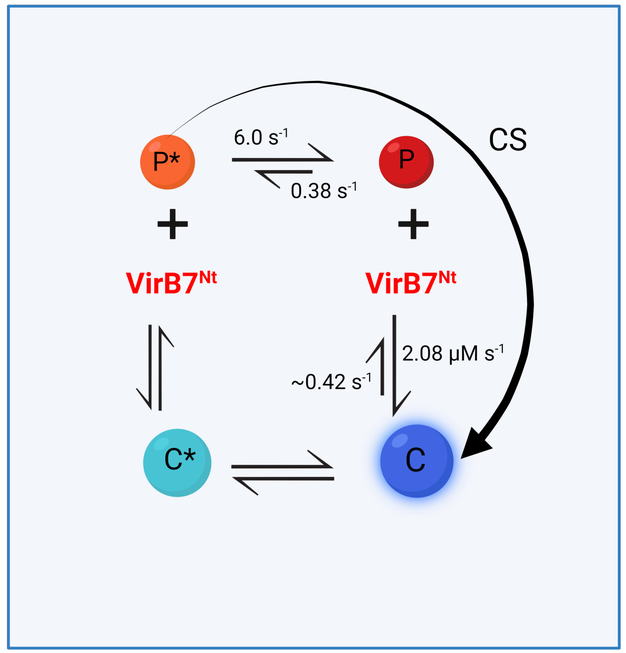


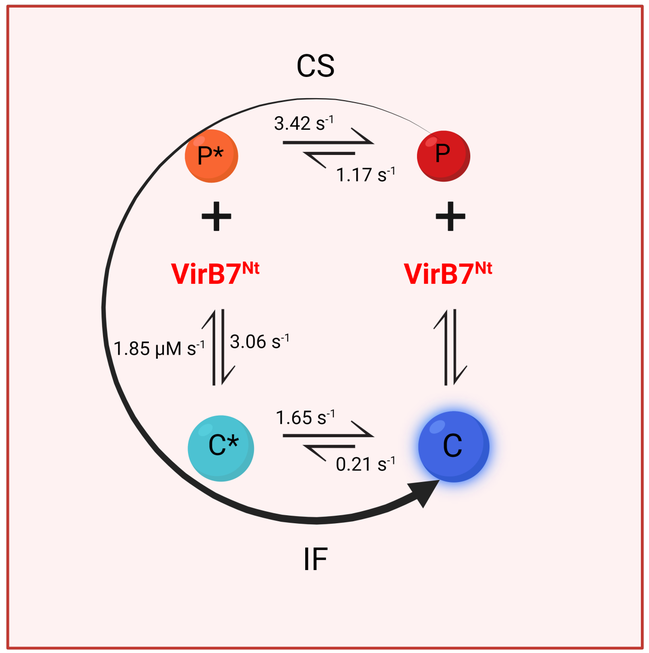


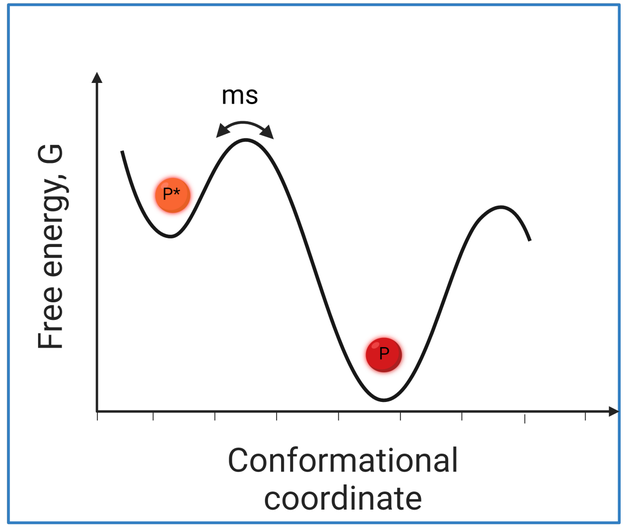
Binding follows a conformational-selection mechanism, however an additional event is observed under excess of VirB9^{CT}

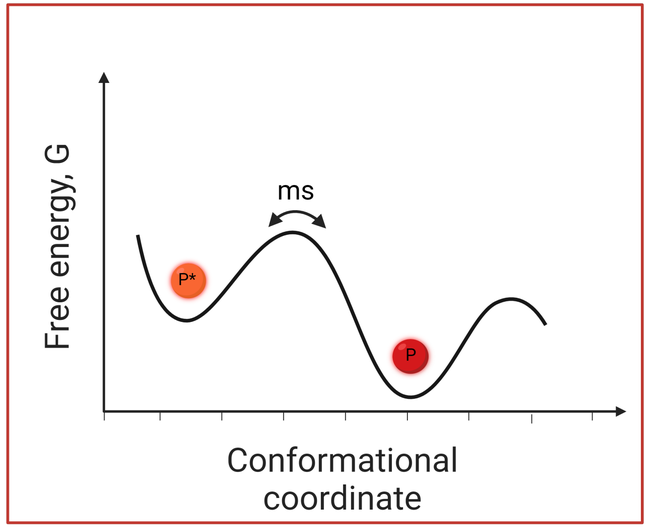
25°C

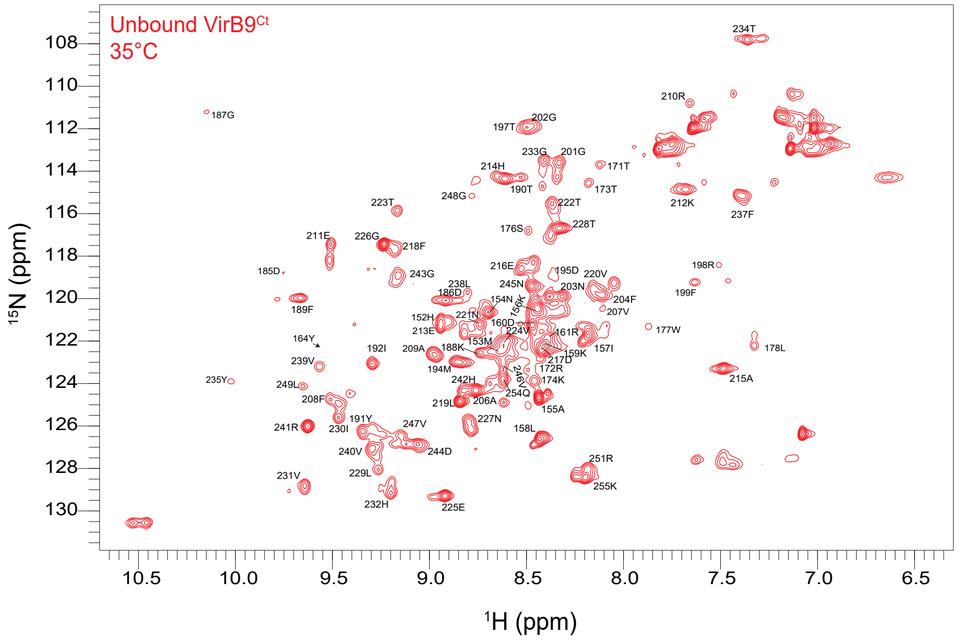
35°C

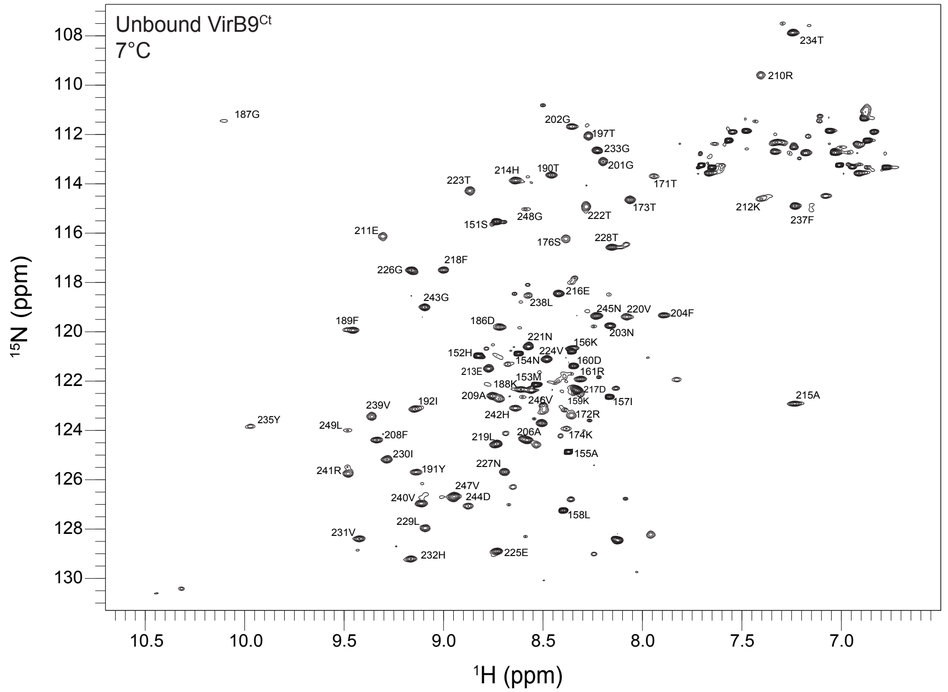

P*
P
6.0 s-1
0.38 s-1
CS
VirB7Nt
VirB7Nt
2.08 μM s-1
~0.42 s-1
C*
C


CS
3.42 s-1
1.17 s-1
P*
P
VirB7Nt
VirB7Nt
1.85 μM s-1
3.06 s-1
C*
1.65 s-1
0.21 s-1
C
IF

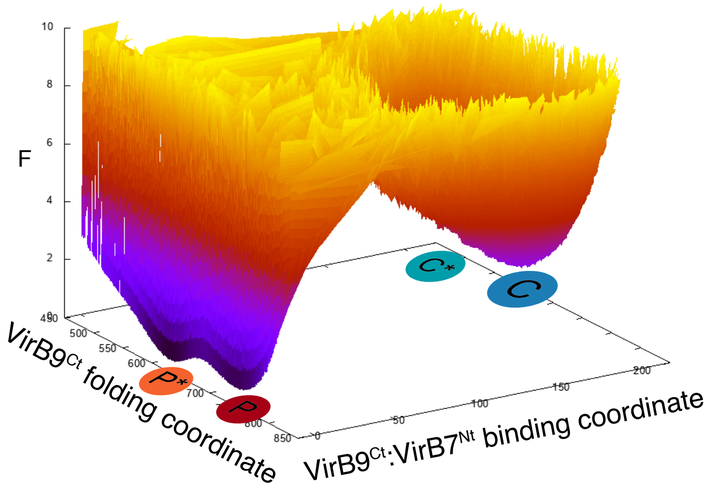

ms
Free energy, G
P*
P
Conformational coordinate

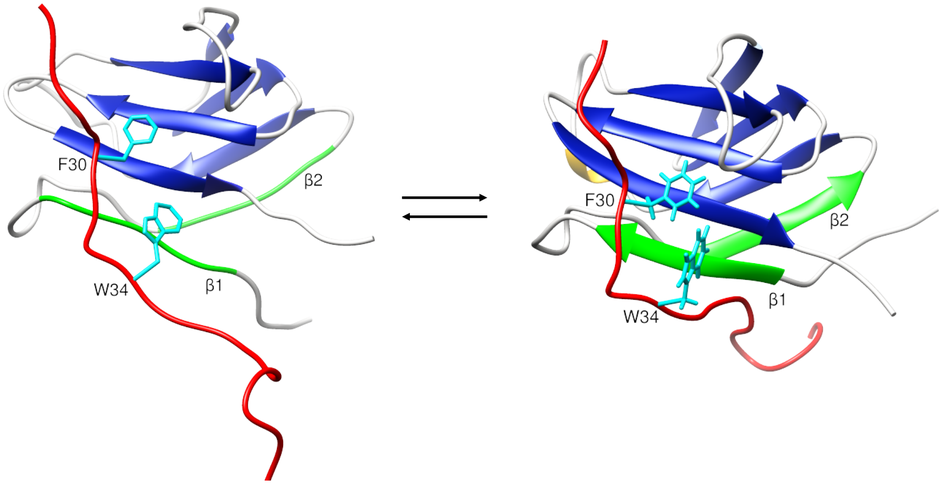

ms
Free energy, G
P*
P
Conformational coordinate

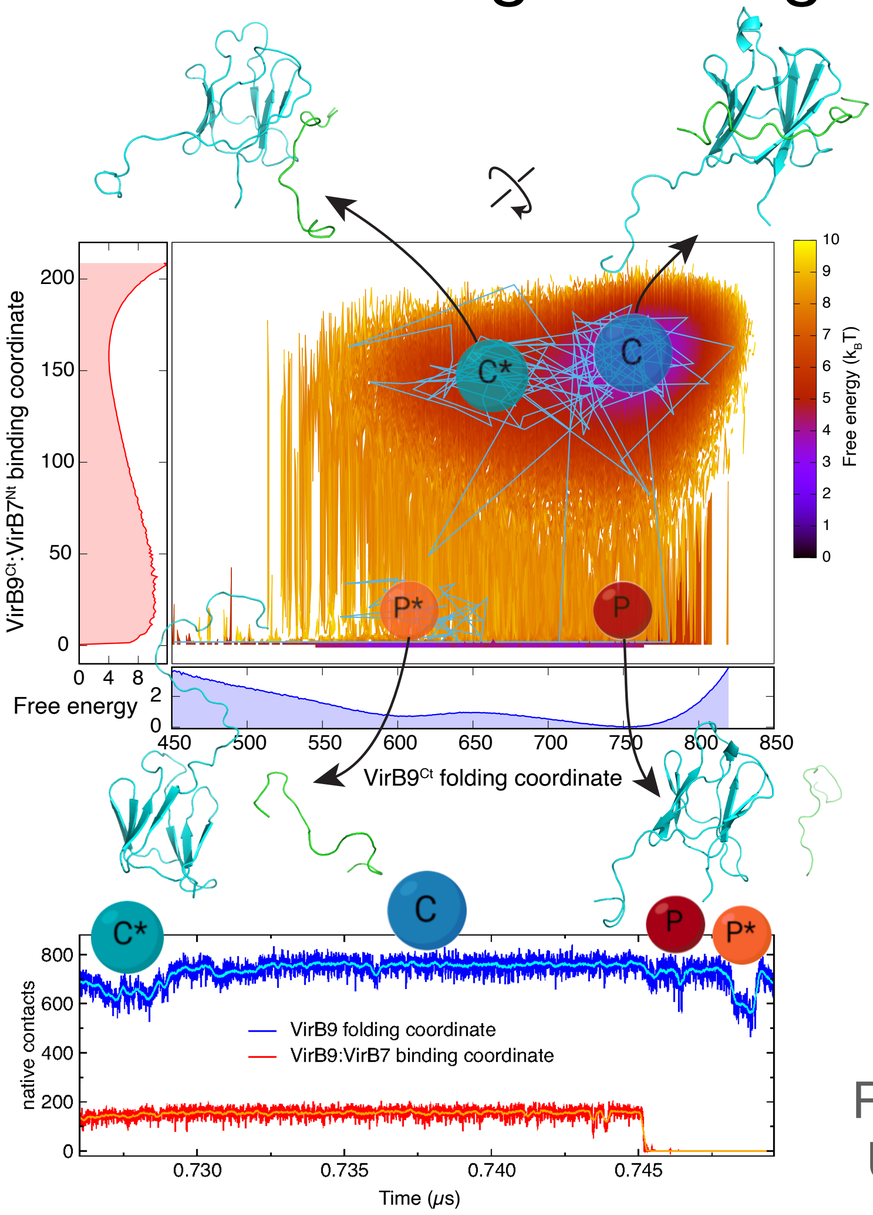

Unbound VirB9Ct
35°C
15N (ppm)
1H (ppm)


Unbound VirB9Ct
7°C
15N (ppm)
1H (ppm)

Modeling the binding association mechanism using coarse grained simulations







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Conclusions

- In the absence of VirB7^{NT}, VirB9^{CT} β1 unfolds
- Binding follows a conformational selection mechanism at 25 ℃
- At higher temperatures, the populations of other conformational states increase, favoring alternative binding pathways and leading to an encounter complex that subsequently folds to the native complex
- Binding involves a downhill trajectory on the funneled energy landscape as the two proteins search for the best intermolecular contacts

Acknowledgements

- Angie Dàvalos (IQUSP)
- Dr. José David Rivera Echeverri (IQUSP)
- Denize C. Favaro (NYU)
- Iolanda Cuccovia (IQUSP)
- Chuck Farah (IQUSP)
- Ronaldo Junio (UFTM)

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