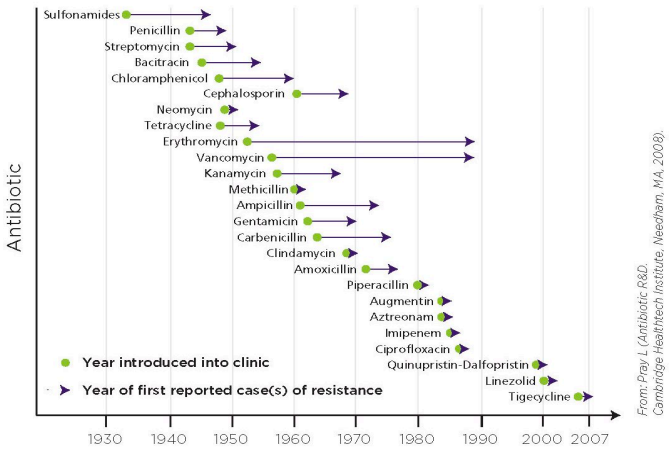
Structure and Function of a Bacterial Type IV Secretion System (T4SS)

Gram-positive Conjugation

InnoMol - Workshop
2014 – 04 – 07

Walter Keller

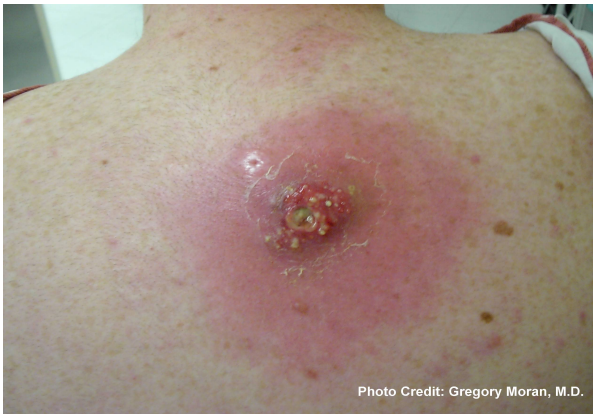
Antibiotic resistance – an arms race



Note: *Some of the dates are estimates only.*

Pray, L. (2008) Antibiotic R&D: resolving the paradox between unmet medical need and commercial incentive. *http://www.nps.org.au/medicines/infections-and-infestations/...*

MRSA – A rising problem in our hospitals



Horizontal gene transfer

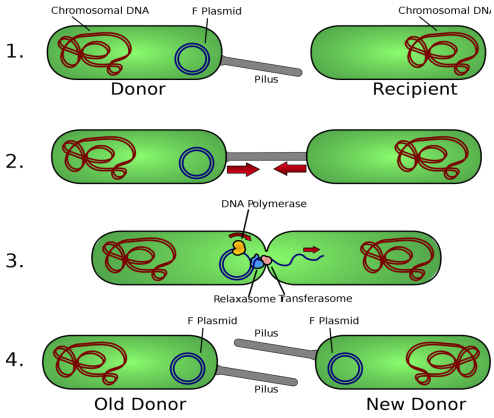
• **Transformation**
Uptake and expression of **foreign genetic material** – may lead to genetic alteration of the cell

• **Transduction**
Caused by **bacterial viruses** (bacteriophages) – **transfer of DNA** between microorganisms – material may be integrated into the host chromosome

• **Gene transfer agents** of *Rhodobacterales*

• **Bacterial Conjugation**
Most prevalent means of DNA transfer – involves **cell-to-cell contact** – utilizes a **multi-protein complex** usually encoded on the transferred plasmid or integrative conjugative element (ICE, transposon)

Bacterial Conjugation (Type-IV Secretion)



From Wikipedia entry „Conjugation“
http://en.wikipedia.org/wiki/File:Conjugation.svg – user: Adenosine (2009)

1. **Donor cell produces pilus**
2. **Pilus attaches to the recipient cell** – cells are brought together – a mating pore is formed
3. **Processing and transfer of the DNA** – the plasmid is nicked and the single-stranded DNA is transferred through the mating pore
4. **Synthesis of the complementary strand** – both cells are viable donors now

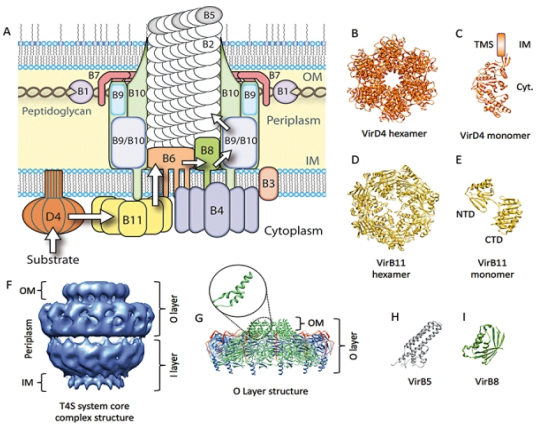
A Gram-negative model: the VirB/D T4SS

Gram-negative T4SS are relatively well understood

Several proteins have been crystallized – in 2009 even the structure of the outer membrane complex got solved

Key components:

- **ATPases** – to provide the necessary energy
- **Relaxases** (+ **auxiliary proteins**) – to process the plasmid DNA
- **Coupling proteins** – to link the relaxase-DNA complex with the conjugative pore
- **Muramidases** – to locally disrupt the bacterial cell wall



Wallden, K. , Rivera-Calzada, and Waksman, G. (2010) Microreview: Type IV secretion systems: versality and diversity in function. *Cell Microbiol.* **12**, 1203-1212

G- vs. G+: Differences and Similarities

Differences...

- Vastly **diverse cell envelope** composition
  → **No** need of an **equally large core-complex** – only one membrane
  → More **prominent role of muramidases** – significantly thicker peptido glycan layer

- **No pili** encoded in Gram-positive T4SS
  → Cell-to-cell contact & surface attachment based on a wastly different mechanism
  → Candidate proteins found in many systems

Similarities...

- **Key transfer proteins are conserved** across T4SS (ATPase, muramidase, ...)

- Only **limited** amount of proteins with **sequence similarities**
  → Does not exclude yet undetected structural similarities...

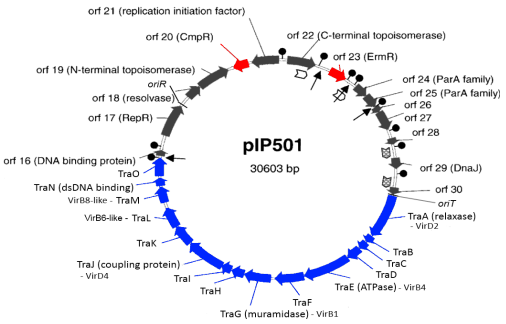
Conjugative Plasmid pIP501

- **30.6 kbp** / first isolated from *Streptococcus agalactiae* / studied in *Enterococcus faecalis*

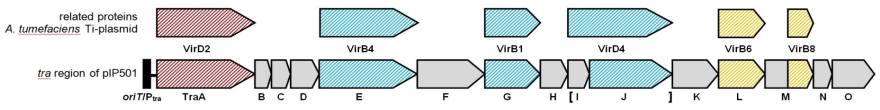
- **Broad host range** (even G- bacteria)

- Encodes for antibiotic resistance genes against **Chloramphenicol & Erythromycin**

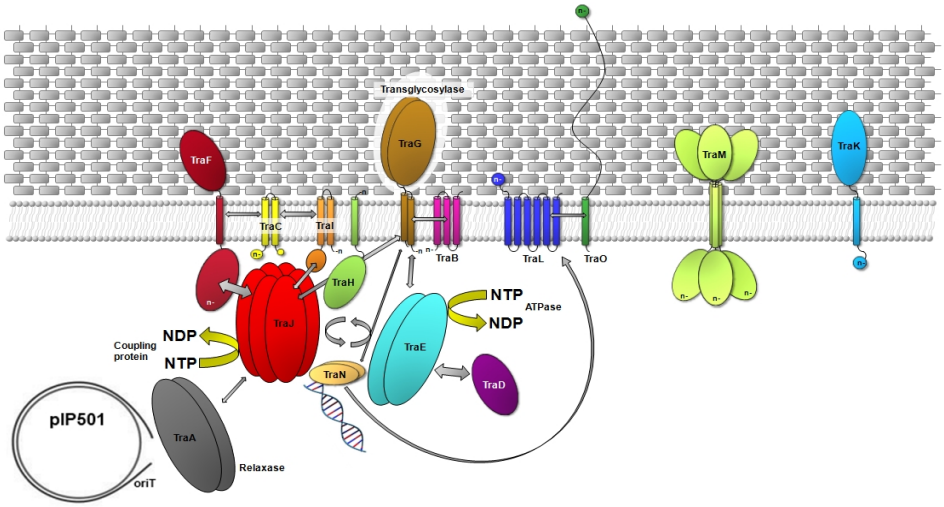
- The **15 transfer proteins** are organized in a **single operon**



Modified from: Thompson, J. And Collins, M. (2003) Completed sequence of plasmid pIP501 and origin of spontaneous deletion derivatives. *Plasmid*. **50**, 28-35

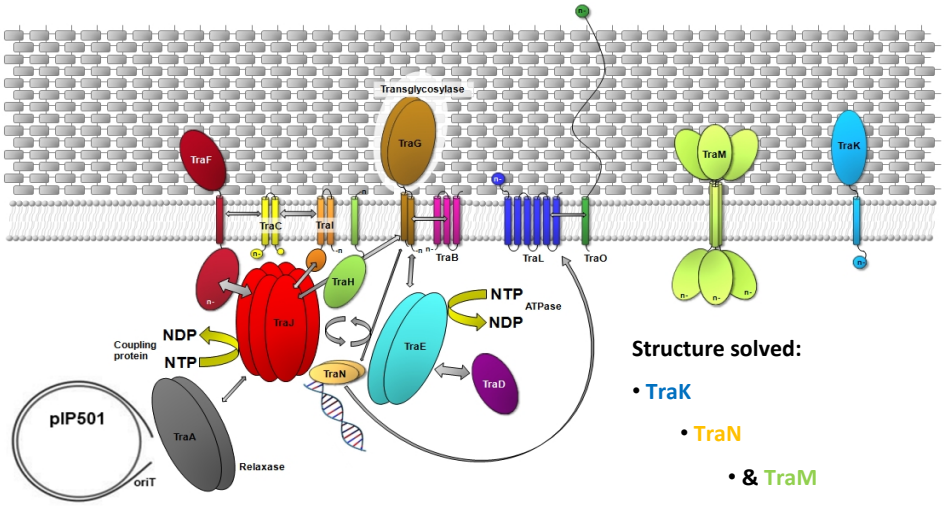


A Preliminary Model



Interactions (arrows) are based on Y2H and pull-down experiments described in: Abajy, M.Y. et al. (2007) A Type IV-Secretion-Like System is Required for Conjugative DNA Transport of Broad-Host-Range Plasmid pP501 in Gram-Positive Bacteria. *J. Bacteriol.* **189**, 2487-2496

A Preliminary Model



Structure solved:

- **TraK**
- **TraN**
- **& TraM**

Interactions (arrows) are based on Y2H and pull-down experiments described in: Abajy, M.Y. et al. (2007) A Type IV-Secretion-Like System is Required for Conjugative DNA Transport of Broad-Host-Range Plasmid pP501 in Gram-Positive Bacteria. *J. Bacteriol.* **189**, 2487-2496

TraM

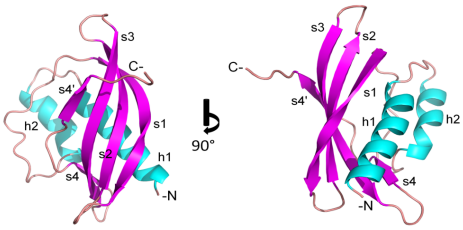
TraM

TraM - Overview

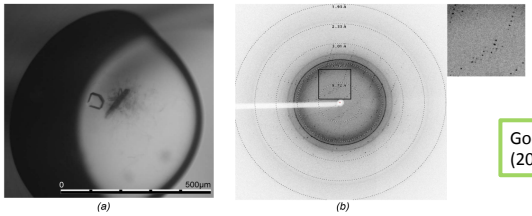
N-terminally truncated construct
transmembrane helix & yet uncharacterized N-terminal domain have been removed

18.6 kDa (including HisTag)

Solubility optimized:
25 mM Hepes pH 7.6, 75 mM $(NH_4)_2SO_4$



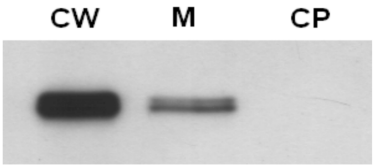
TraMΔ was **successfully** purified, biophysically characterized and crystallized, as well as had its structure **solved** by **Lukas Grumet**



Goessweiner-Mohr, N., Grumet, L., et al. (2013) *Acta Crystallogr F.* **69**, 178–183

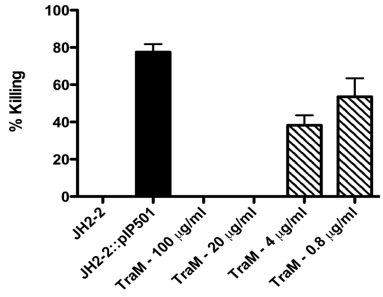
TraM - Localization

TraM localizes to the cell envelope
of pIP501 harboring *E. faecalis* cells



The opsonophagocytic killing assay showed **killing of pIP501 harboring cells** & dose dependent **inhibition of killing**

→ **TraM is surface exposed**



Localization assay performed by PhD Karsten Arends (TU Berlin); Opsonophagocytic killing assay performed by Andrea Kropec-Hübner (Medical University Freiburg)

TraM – Structurally Related Proteins

Structural similarity search using **DALI & MATRAS:**

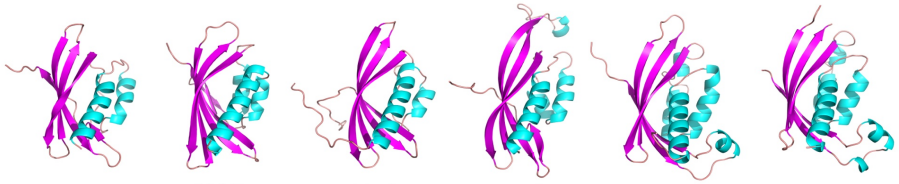
→ **surprising hits from Gram-positive / -negative T4SS**
→ **VirB8** from **G- *A. tumefaciens*** and ***Brucella suis***
→ **TcpC** from **G+ *Clostridium perfringens*** (pCW3)

but

A

TraMΔ	Sequence identity [%]	Secondary structure similarity [%]	Superfamily reliability [%]	Fold reliability [%]
NTF2	8.1	74.7	91.9	98.6
TcpC C-terminal	18.2	87.9	93.6	98.7
TcpC central	13.9	82.4	95.5	99.0
VirB8 *A.t.*	5.8	76.0	82.6	97.8
VirB8 *B.s.*	5.9	77.2	87.9	98.4

→ **very low sequence identity**
→ **similar domains BUT wastly different overall structural composition**



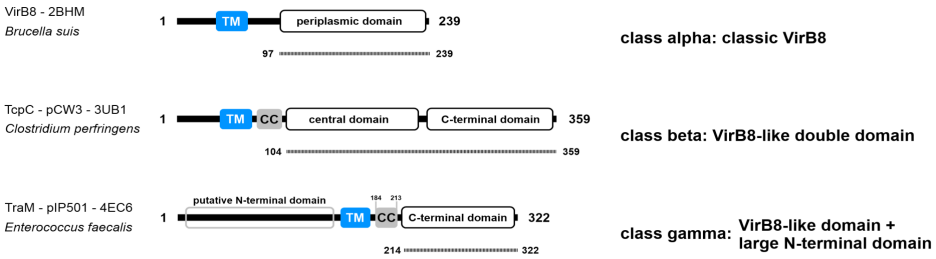
TraM – Other TraM-like Proteins

Secondary structure based search (as for TraK / TraN)

A **vast number** of VirB8-like / TraM-like proteins **found**

The **proteins found** could be **classified** based on their **structural composition**

Class gamma proteins (TraM-like) only found in ***E. faecalis*** **conjugative plasmids**



Goessweiner-Mohr, N., Grumet, L., et al. (2013) The 2.5 A Structure of the Enterococcus Conjugation Protein TraM resembles VirB8 Type IV Secretion Proteins. *Journal of Biological Chemistry.* **288**, 2018–2028

TraM - Conclusions

Structurally **striking similarities** to other **transfer proteins**

Possible functions of TraM

→ **Similar** to structurally **related proteins** (postulated: **scaffolding factor** of the core complex) ?

→ **Attachment site** for the recipient cell

→ Morphogenesis of the actual **core-complex**

Different overall structural composition points to a **different task** in T4SS

Very limited number of structurally **„identical" proteins**

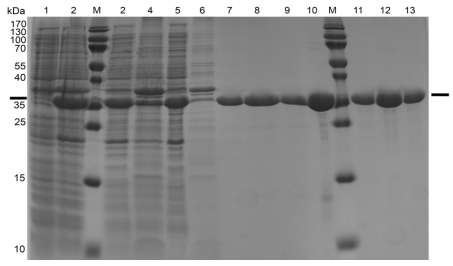
→ **exclusive / specialized role** of the protein in these T4SS

TraK

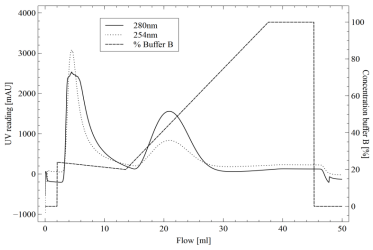
N-terminally truncated construct
transmembrane helix has been removed
to make the protein soluble

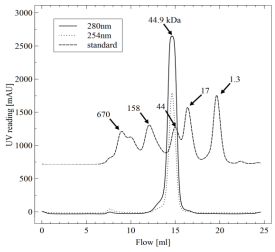
30.6 kDa (including HisTag)

Solubility optimized (Thermofluor method):
25 mM Hepes pH 7.6, 75 mM Na_2SO_4



TraK was successfully purified via affinity and size-exclusion chromatography



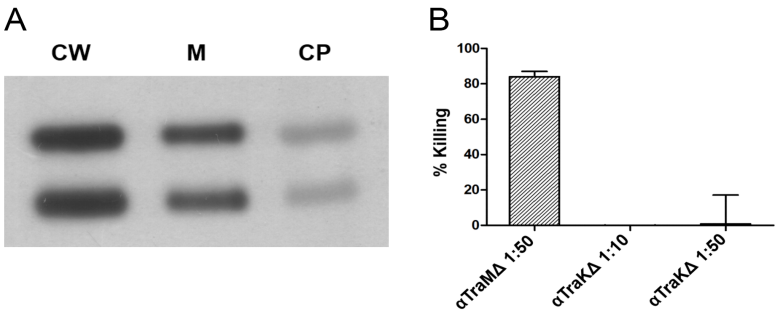


TraK - Localization

TraK localizes to the cell envelope of pIP501 harboring *E. faecalis* cells

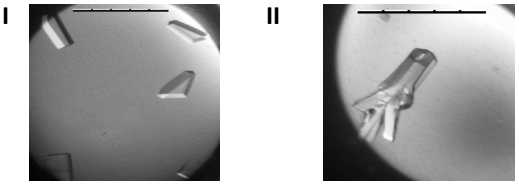
Double signal → possible second start codon within the TraK sequence

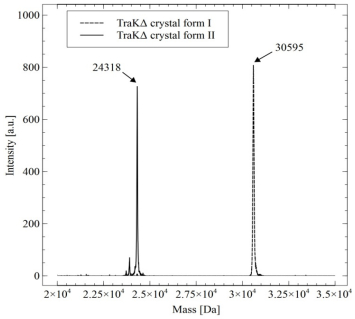
The opsonophagocytic killing assay showed no killing → **TraK is not surface exposed**



Localization assay performed by PhD Karsten Arends (TU Berlin); Opsonophagocytic killing assay performed by Andrea Kropec-Hübner (Medical University Freiburg)

TraK - Crystals



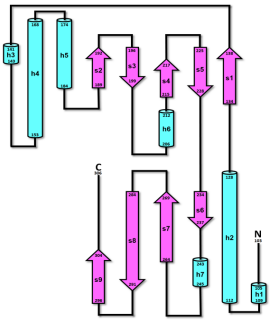


MS experiments performed by Prof. Ruth Birner-Grünberger (ZMF Graz)

Crystal form I – I4 – Morepheus 85
a = b = 114.04, c = 120.52, α = β = γ = 90°
2 molecules / **AsU**; solvent content: **61.6 %**
Resolution @ home: ~6 Å / Resolution @ **SLS: ~3 Å**
Structure solved with **full MAD data set**

Crystal form II – $P2_1$ – Index 85
a = 61.94, b = 197.05, c = 164.12, β = 91.04°
Most likely **18 molecules** / **AsU**; solvent content: **46.2 %**
Resolution @ home: ~5 Å / Resolution @ **SLS: ~2.5 Å**
Data can not be refined → no electron density for 6 of the molecules

TraK – The Structure

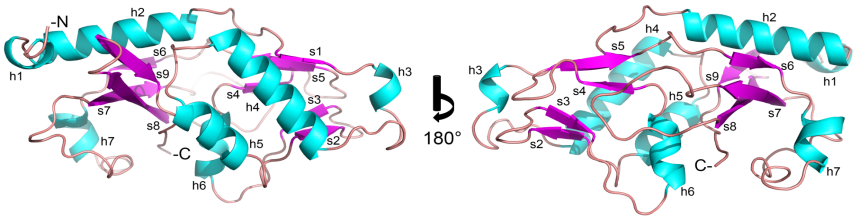


HisTag + 37 N-terminal residues are **not visible** in the electron density map

TraKΔ comprises a **mixed alpha-beta fold**
→ 7 helices & 9 beta-strands

Beta-strands form **2 beta-sheets** - mostly protected from solvent content by helices and loops

Structural similarity search using **DALI & MATRAS:**
→ **no significant hits**



TraK – Other TraK-like Proteins

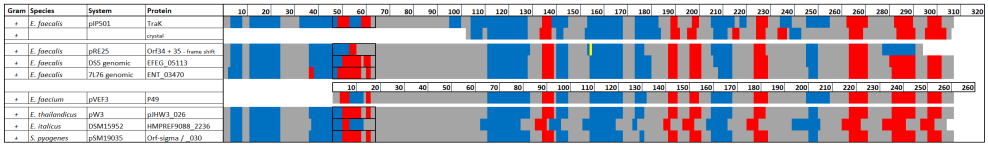
Secondary structure based search for related proteins in a large number of **Gram-positive** and **–negative** conjugative plasmids, genomic islands (GIs), transposable elements and integrated conjugative elements (ICEs)

Only **very limited amount** of TraK-like proteins

Mostly from *Enterococcus* species (one exception: *Streptococcus pyogenes*)

Nearly identical secondary structure composition

→ **related systems !?**



TraK - Conclusions

ssDNA interaction

→ TraK might be involved in the **transport of the single stranded plasmid**

Localization at the **cell envelope but not surface exposed**

→ TraK might be an **integral component** of the **core complex**

or

→ TraK is **not positioned** near the **opening in the peptidoglycan layer**

Very limited number of structurally **related proteins**

→ **exclusive / specialized role** of the protein in these T4SS

TraN

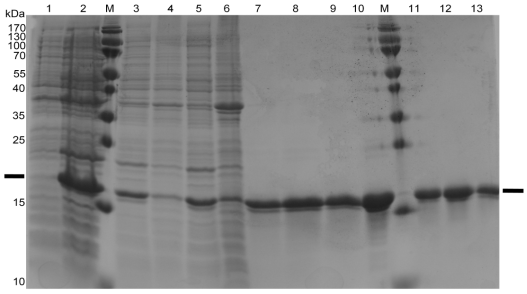
TraN

TraN - Overview

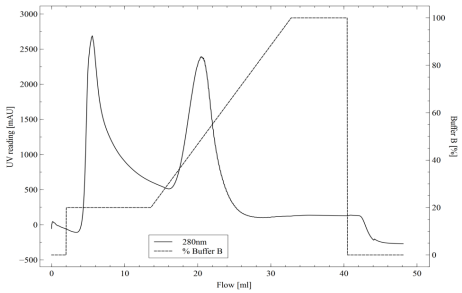
17.6 kDa (including HisTag)

Solubility optimized:
25 mM Hepes pH 7.6, 75 mM Na_2SO_4

TraN was successfully purified via affinity and size-exclusion chromatography



(a)



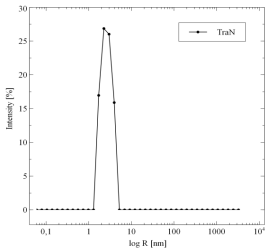
(b)

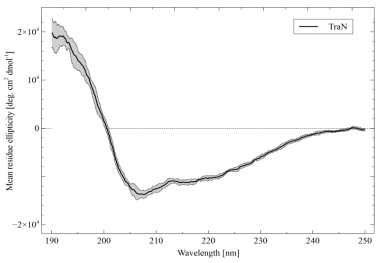
TraN - Characterization

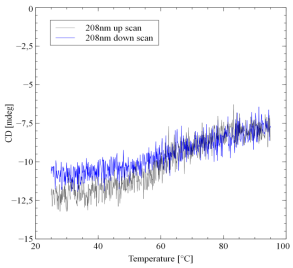
TraN is a **monomer in solution**

TraN is **folded in solution** with about 34 % alpha-helices & 19 % beta-strands

In the optimized buffer, **TraN** has a **melting temperature** of about **60 °C** and is **able to refold** nearly completely

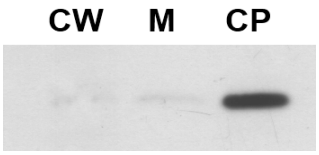






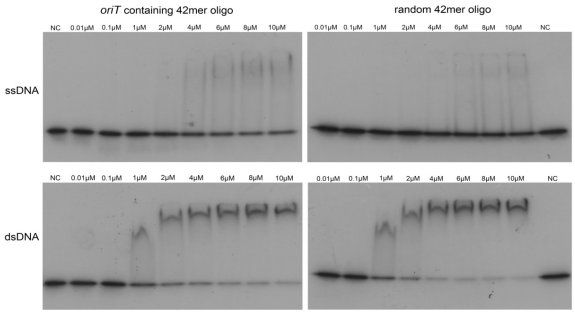
TraN – Localization & DNA Interaction

TraN localizes to the cytoplasm
of pIP501 harboring *E. faecalis* cells



TraN interacts with DNA

- **no preference** for the pIP501 **oriT sequence**
- interaction significantly stronger for **dsDNA**

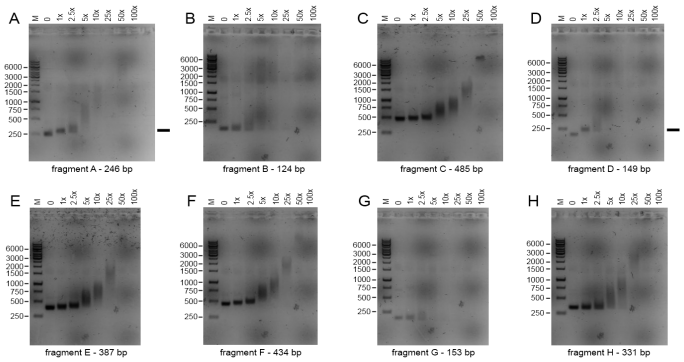


Localization & EMSA assays performed by PhD Karsten Arends (TU Berlin)

TraN – Localization of the DNA Binding Site (BS)

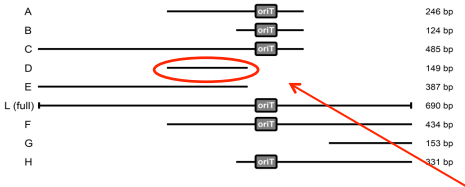
New band-shift assays

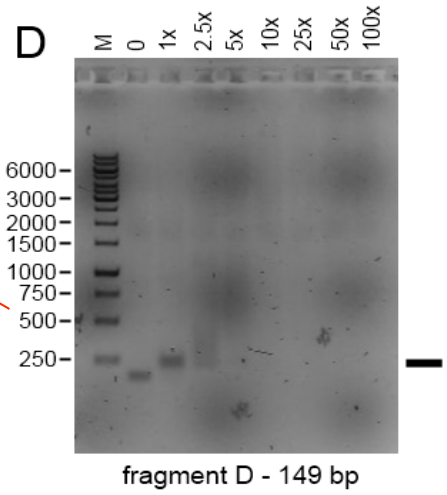
TraN binds to dsDNA in general
→ co-operative shift of all fragments with higher TraN conc.



TraN – Localization of the DNA Binding Site (BS)

Distinct shift for fragment D (and others)



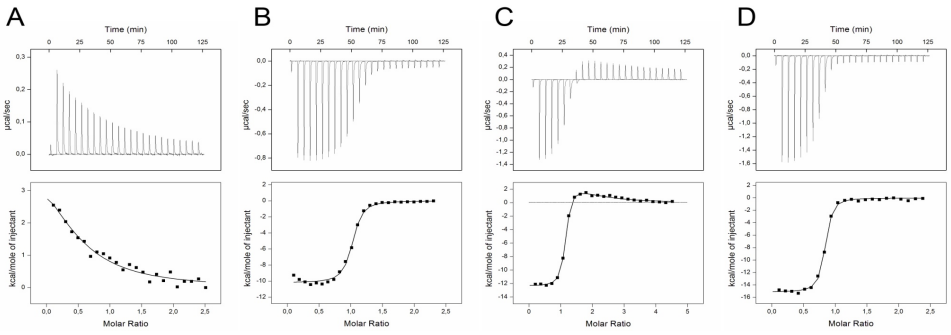


Binding site identified
→ newly developed method (preliminary data)

Results will be confirmed
via a conventional **footprinting assay**
(found to be quite tricky)

TraN – Confirmation of the TraN Binding Site (BS)

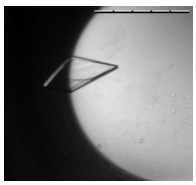
ITC experiments with the specific BS and random DNA

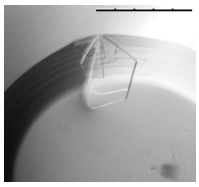


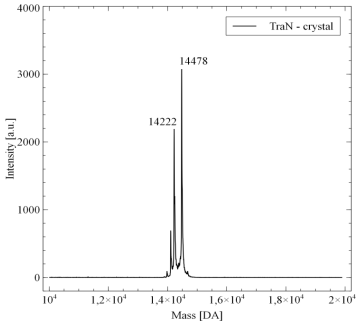
random DNA | specific BS | reverse specific BS | specific BS vs. random DNA

Random interaction:	endothermic	protein : DNA = 2 : 1	k: 10^5
Specific interaction:	exothermic	protein : DNA = 1 : 1	k: 10^7

TraN - Crystals

I 

II 



MS experiments performed by Prof. Ruth Birner-Grünberger

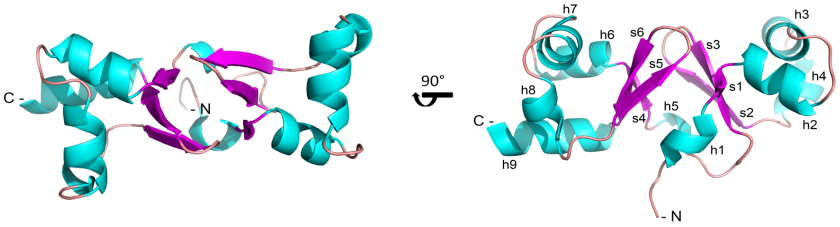
Crystal form I – $P2_1$ – Index 72
a = 33.15, b = 55.09, c = 35,52, β = 113.05°
1 molecule / **AsU**; solvent content: **39.9 %**
Resolution @ home: <2 Å / Resolution @ **SLS: 1.35 Å**
Structure solved with **SAD data set**

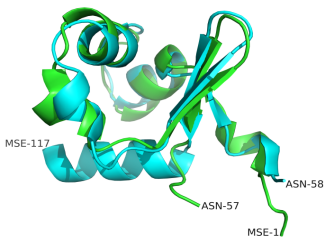
Crystal form II – $P2_1$ – Index 42
a = 33.10, b = 62.82, c = 55.87, β = 90.03°
2 molecules / **AsU**; solvent content: **38.2 %**
Resolution @ home: <2 Å / Resolution @ **SLS: 1.4 Å**

Goessweiner-Mohr, N., Fercher, C., et al. (2012) *Acta Crystallogr F.* **68**, 1402–1405

Crystal form III - $P2_1$ – Index 42 → very similar to crystal form III
→ original native data, 1.8 Å

TraN – The Structure





HisTag is **lost** during crystallization

TraN comprises a **mixed alpha-beta fold**
→ 9 helices & 6 beta-strands

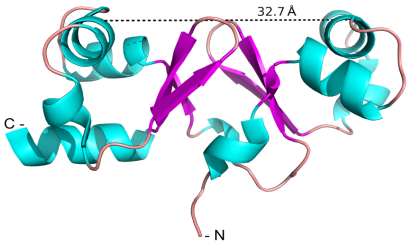
The structure reveals an **internal dimer fold**

Beta-strands form a **„beta-barrel" –like motif** in the middle of the molecule (stabilized by hydrophobic interactions)

TraN – Structural Basis for DNA Interaction

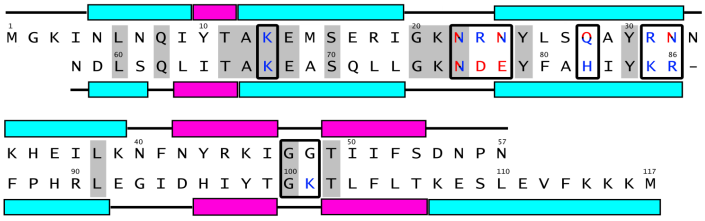
Two „helix-turn-helix“ –like motifs

Two parallel helices in perfect distance for **interaction** with two ajacent **major grooves** of **dsDNA**



The likely **involved residues** are **differently charged**

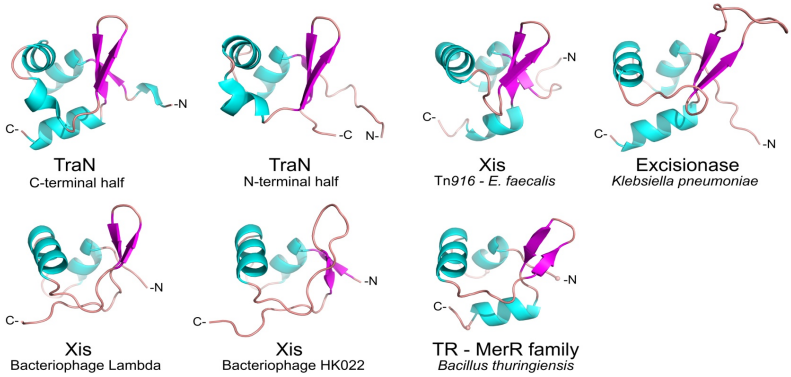
→ **binding site**: presumably **no direct or inverted repeat**



TraN – Structurally Related Proteins

Structural similarity search using **DALI & MATRAS:**

→ **only hits for one half of TraN**
→ **excisionases** from conjugative transposons and bacteriophages
→ another interesting hit: **MerR family of transcriptional regulators**



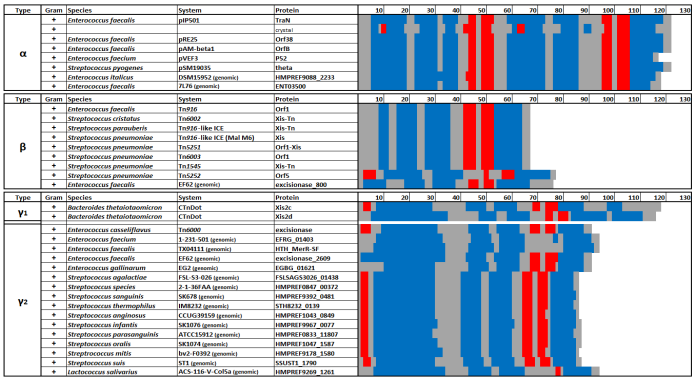
TraN – Other TraN-like Proteins

Secondary structure based search (as for TraK)

Only **very limited amount** of „identical“ proteins

Same hits as for TraK → **related systems !?**

Other proteins are all **excisionases** from Gram-positive transposons & ICEs



TraN - Conclusions

dsDNA interaction

→ TraN might be involved in the **processing of ds plasmid DNA prior to the actual transport**

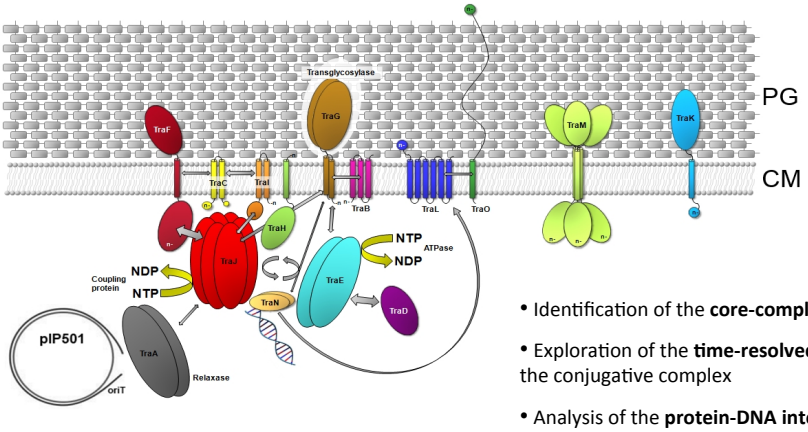
Possible functions:

- **auxiliary protein** for the pIP501 **relaxase TraA**
- **excisionase of the pIP501 T4SS** (rather not)
- **transfer regulator** – protection of the pIP501 plasmid prior to the establishment of cell-to-cell contact

Very limited number of structurally **related proteins**

→ **exclusive / specialized role** of the protein in these T4SS

Still a lot to do ...

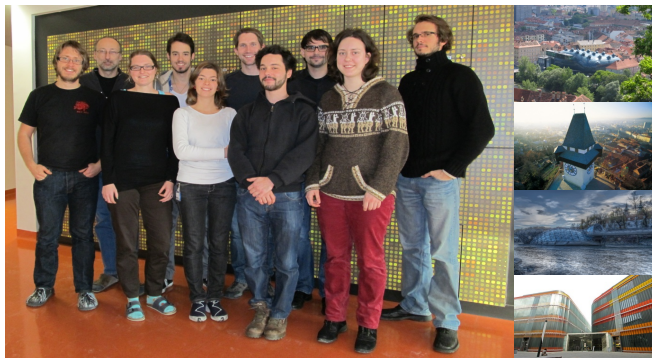


- Identification of the **core-complex components**
- Exploration of the **time-resolved build-up** of the conjugative complex
- Analysis of the **protein-DNA interaction** pathway
- **Knock-out studies** to identify the **essential transfer proteins**

Acknoledgements

Graz (IMB)
Nikolaus Gössweiner-Mohr
Christian Fercher
Lukas Grumet
Ertugrul-Kaan Çelic
Jaser Abaji
Jolanta Kopec

Anđela Đordić
Domen Zafred
Kerstin Fauland
Markus Eder
Gerhard Hofer
Andrea Teufelberger



Cooperations:
Elisabeth Grohmann
Karsten Arends
Johannes Hübner

Ruth Birner-Grünberger (ZMF, MedUni Graz)
Klaus Zangger (IfC, Uni Graz)

Acknoledgements

Freiburg (Medical University) / Berlin (TU-Berlin)

Prof. Elisabeth Grohmann

PhD, Karsten Arends

Dr. Johannes Hübner

Frankfurt (Max-Planck-Institute for Biophysics)

Prof. Werner Kühlbrandt

Dr. Thomas Meier