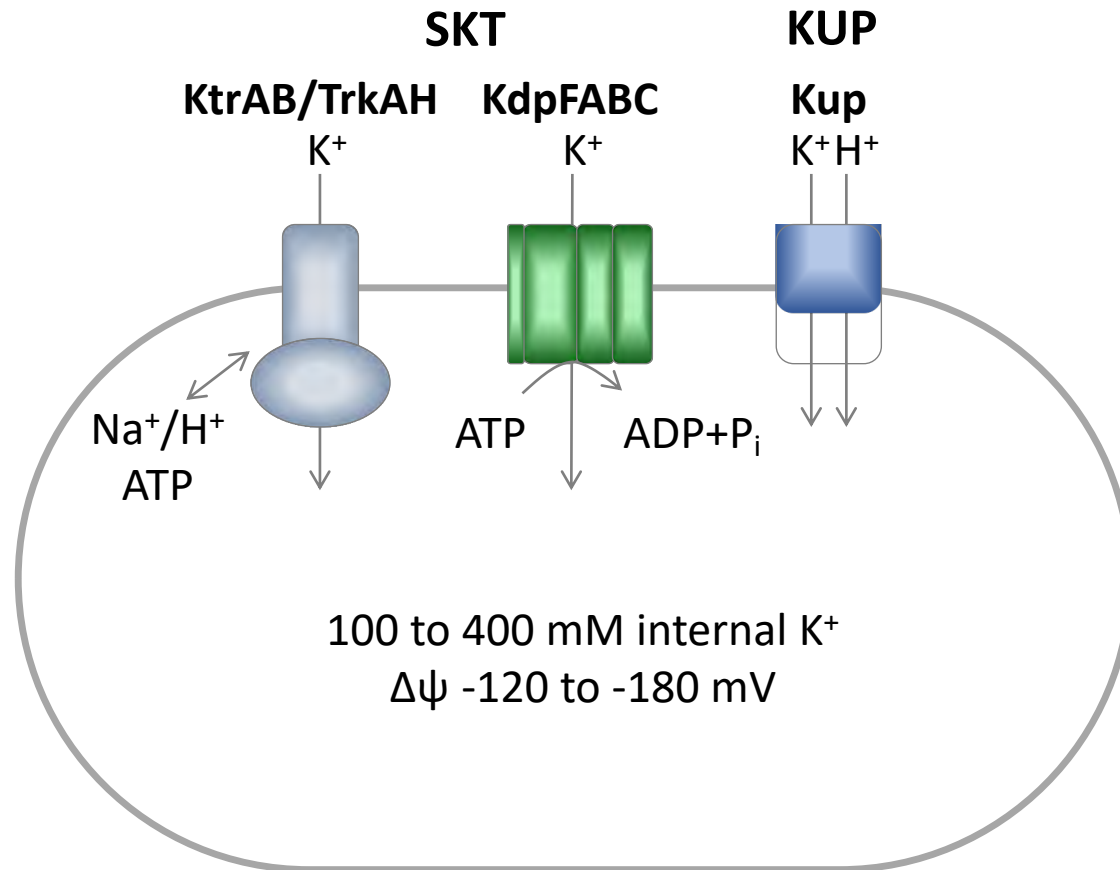


Structural basis of proton-coupled potassium transport in the KUP family

SMALP Zoom meeting 19th June

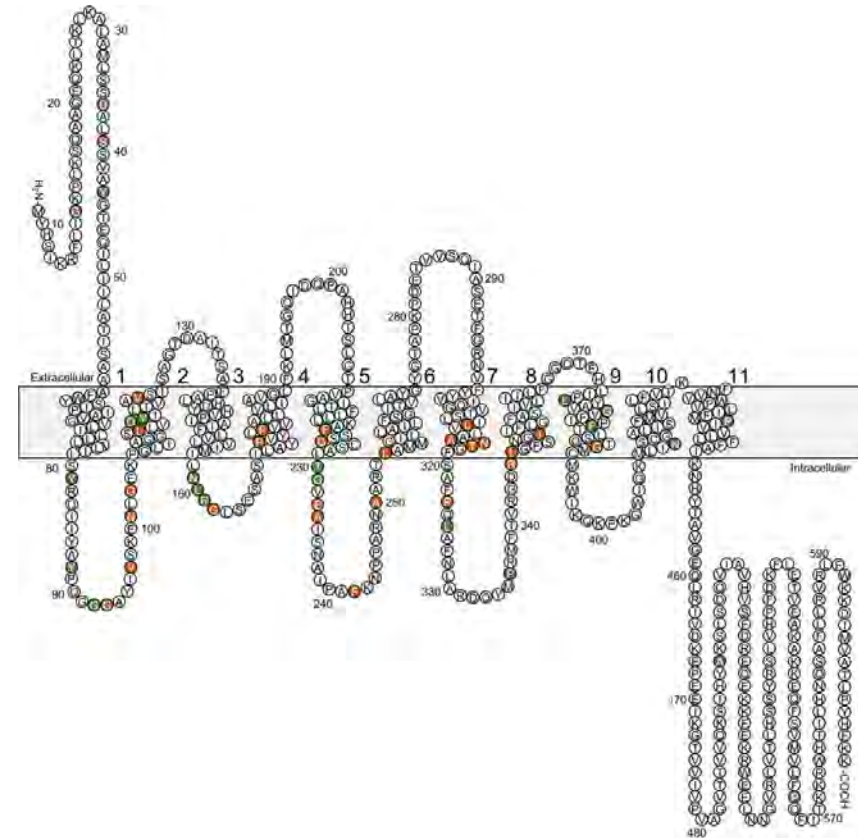
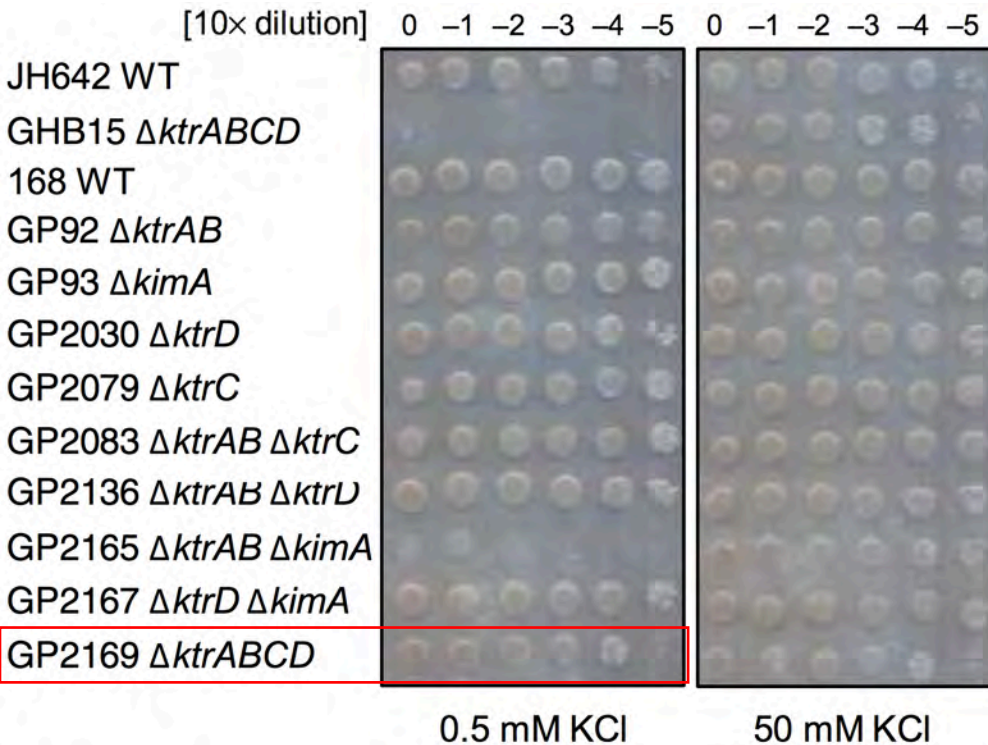
Igor Tascón
Instituto Biofisika (UPV/EHU-CSIC)
Bilbao. Basque Country

Role of K⁺



Osmoadaptation, Intracellular pH regulation
Enzyme activation, Electrical signalling in biofilms

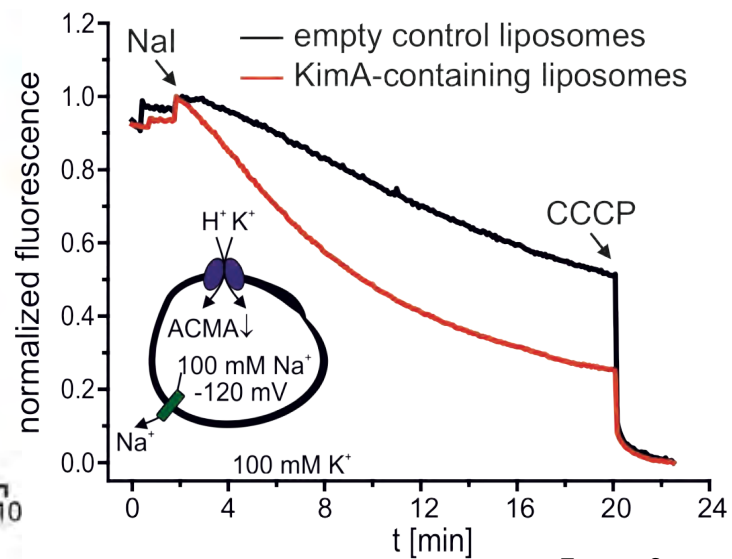
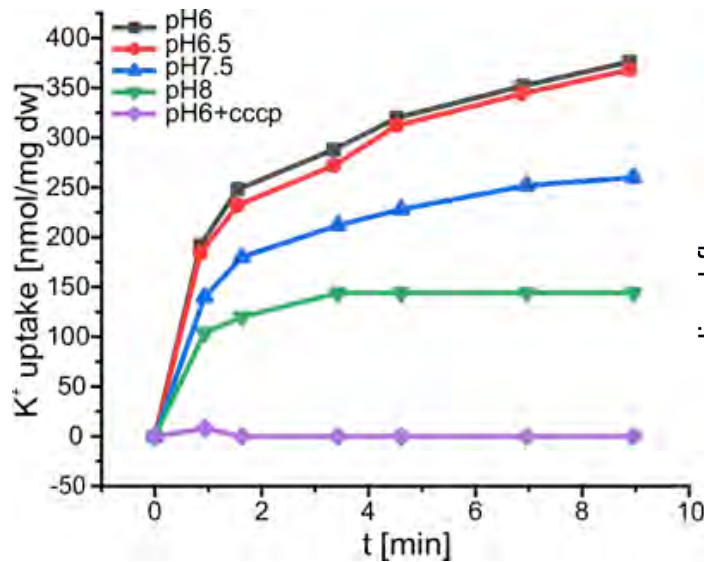
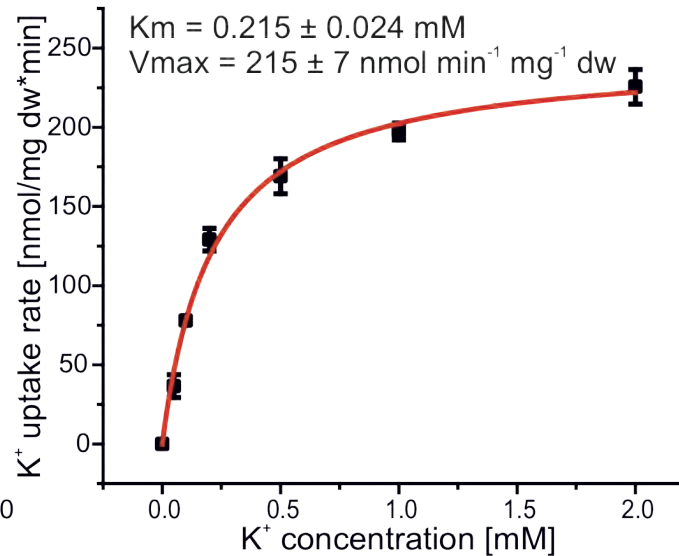
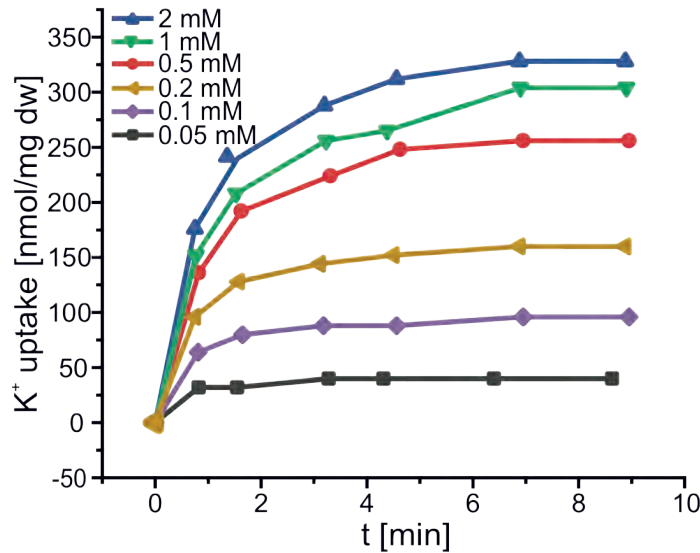
KimA: newly discovered K⁺ uptake system



Gundlach *et al.* Sci Signal, 2017

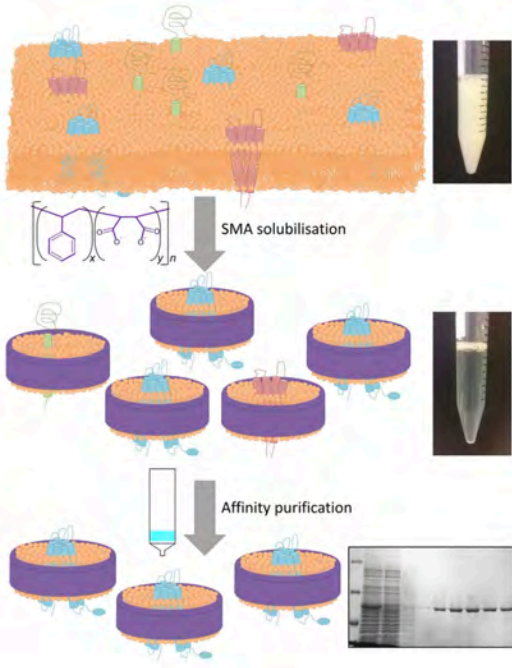
KimA belongs to the APC superfamily
 Is KimA a H⁺/K⁺ symporter?
 What are the structural basis of K⁺ uptake?

Functional study of KimA



KimA functions as a proton-potassium symporter

Co-polymer based KimA extraction



Pollock *et al.*, 2018

| Product | S:MA Ratio† | Mw† (g/mole) | Mn† (g/mole) | Source |
|----------------------------|-------------|--------------|--------------|------------------|
| Xiran® SL 25010 P20 | 3:1 | 10000 | 4000 | Polyscope |
| Xiran® SL 30010 P20 | 2.3:1 | 6500 | 2500 | Polyscope |
| Xiran® SL 40005 P20 | 1.2:1 | 5000 | 2000 | Polyscope |
| Sokalan CP9† | n/a | 15300 | 8400 | BASF |

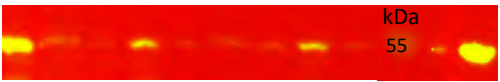
$$\left[\text{Styrene} - \text{maleic acid} \right]_n$$

Styrene maleic acid co-polymer

$$\left[\text{CH}_2 - \text{C}(\text{CH}_3) - \text{CH}(\text{CH}_3) - \text{CH}(\text{COO}^-) - \text{CH}(\text{COO}^-) \right]_n$$

poly(diisobutylene-*alt*-maleic acid), DIBMA

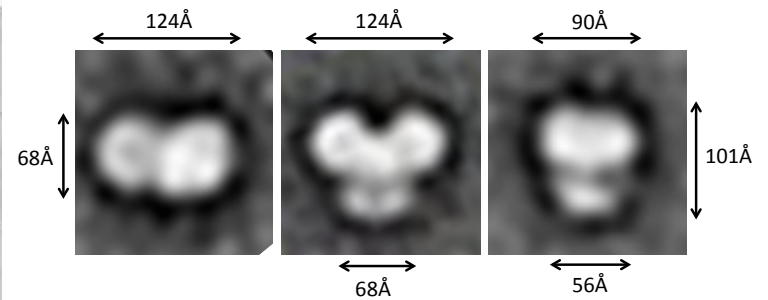
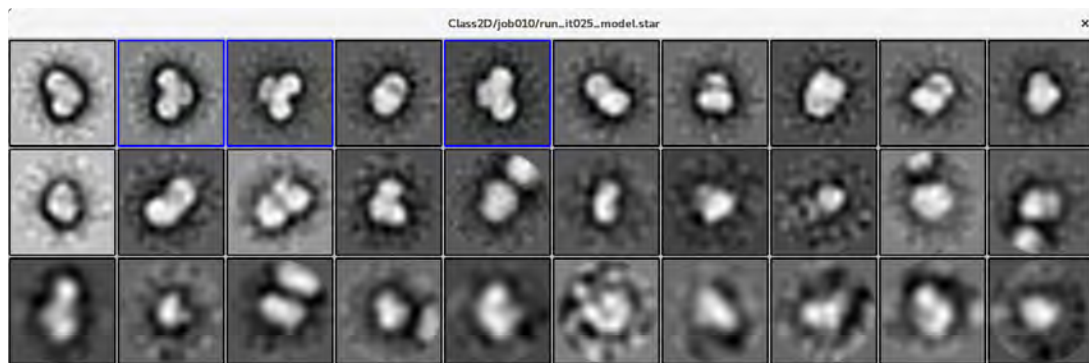
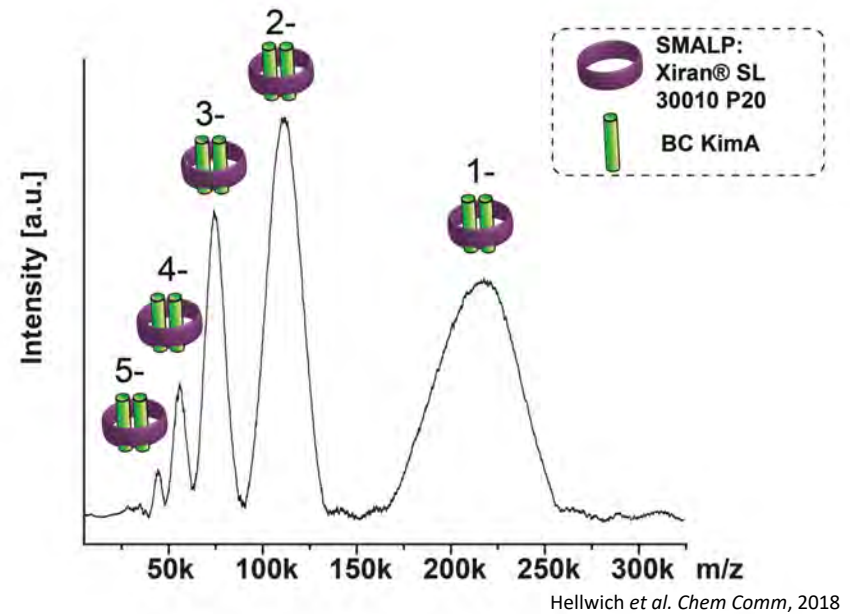
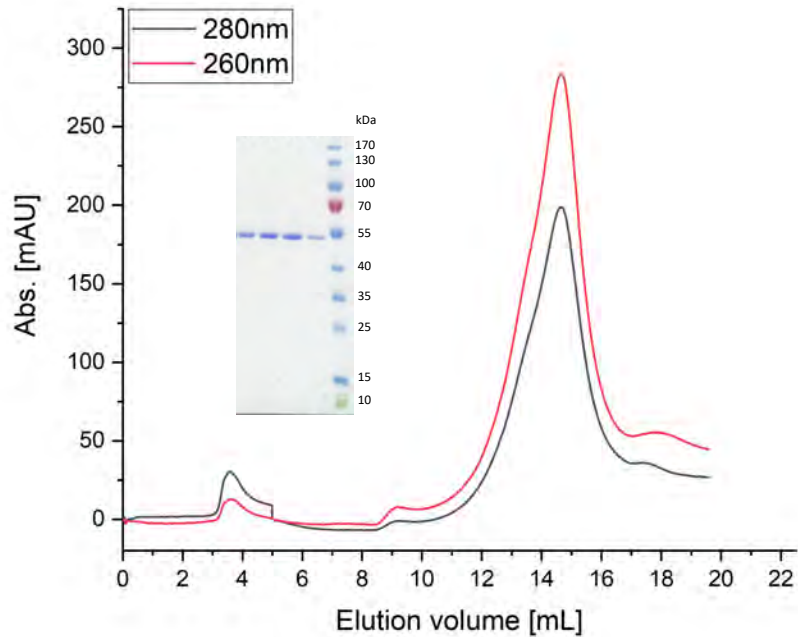
membrPellet
 SN-DIBMA
 SN-X40005
 SN-X30010
 SN-X25010
 FT-DIBMA
 FT-X40005
 FT-X30010
 FT-X25010
 Mw
 E-DIBMA
 E-X30010



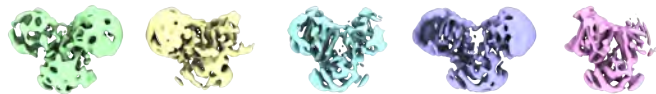
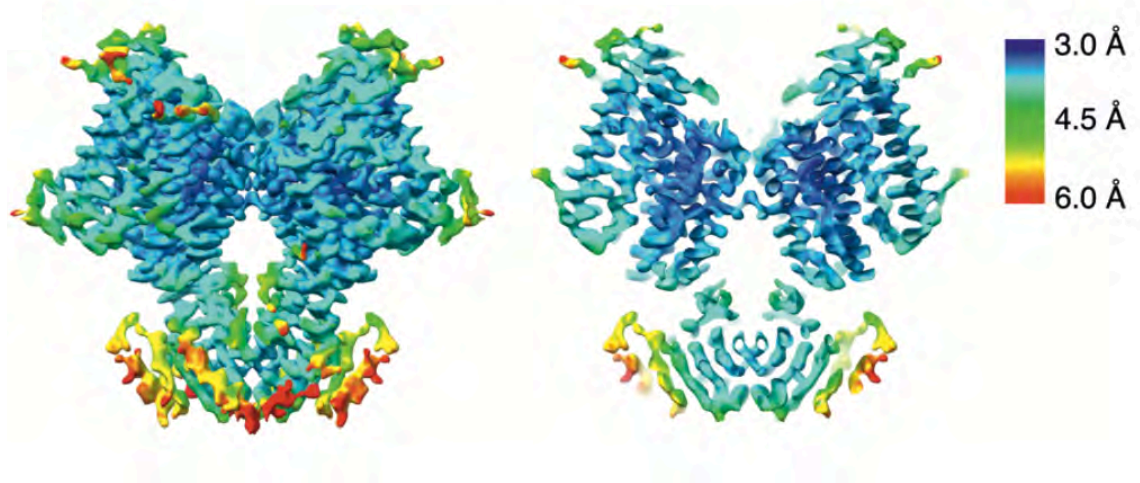
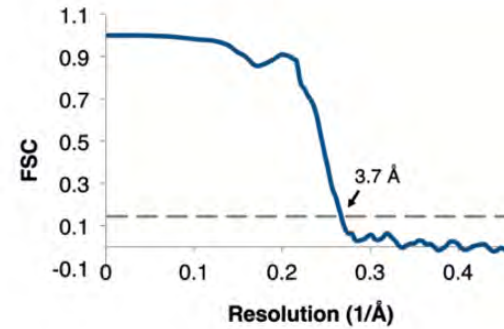
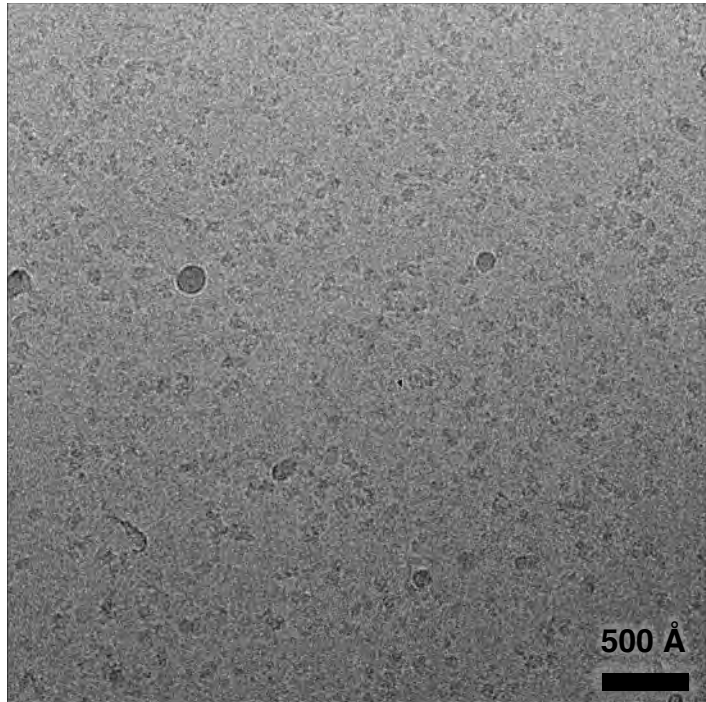
→ KimA-His

WB:antiHis

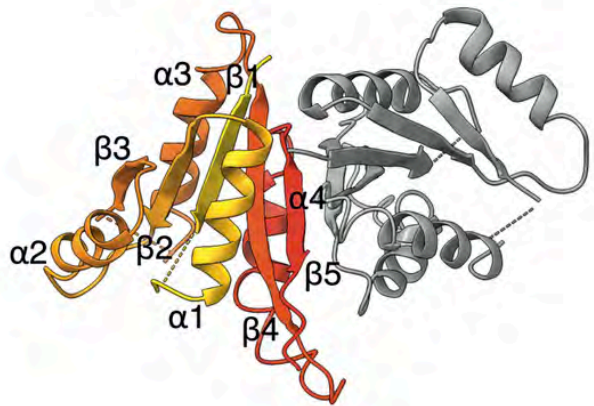
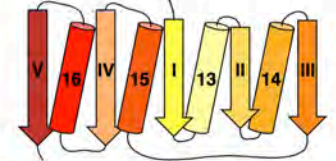
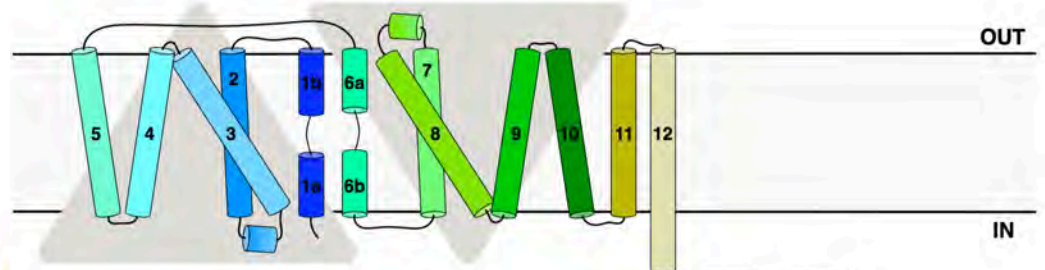
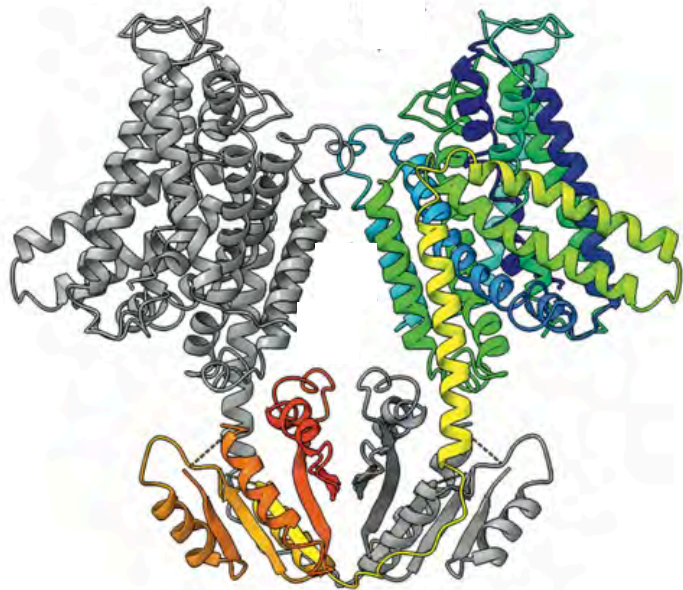
Structural study of KimA in SMALP



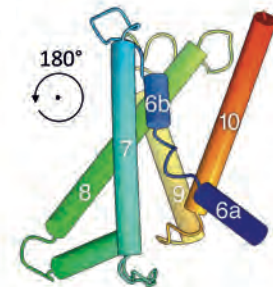
Cryo-EM analysis of KimA in SMALP



KimA overall structure



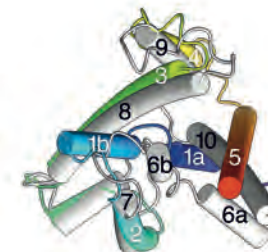
Phosphopantetheine adenylyl transferases folding



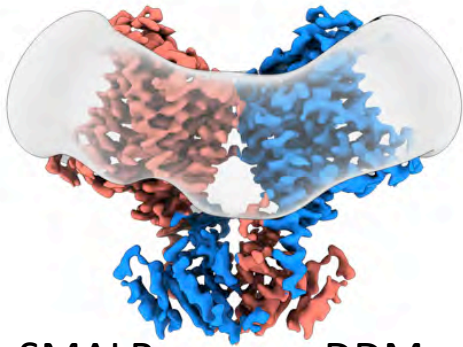
180°



90°

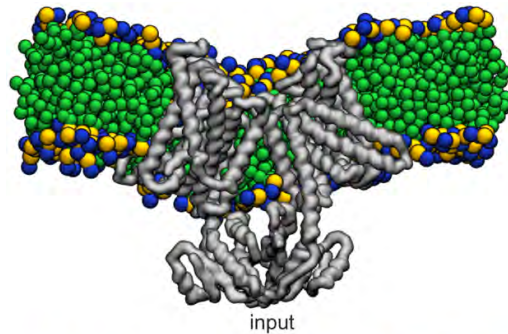
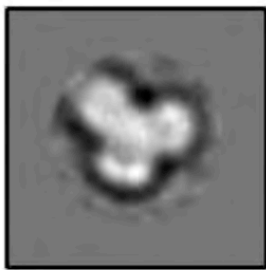
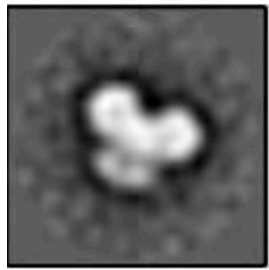


KimA-TM domains interface

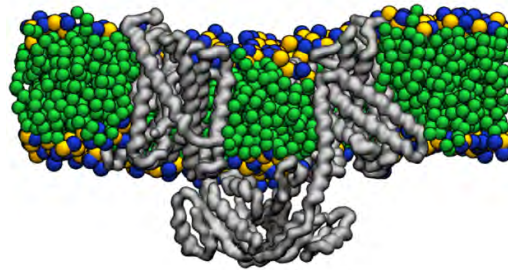


SMALP

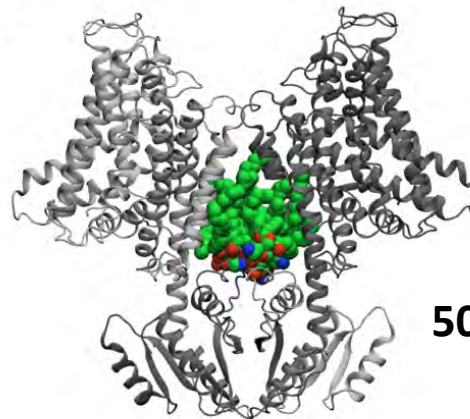
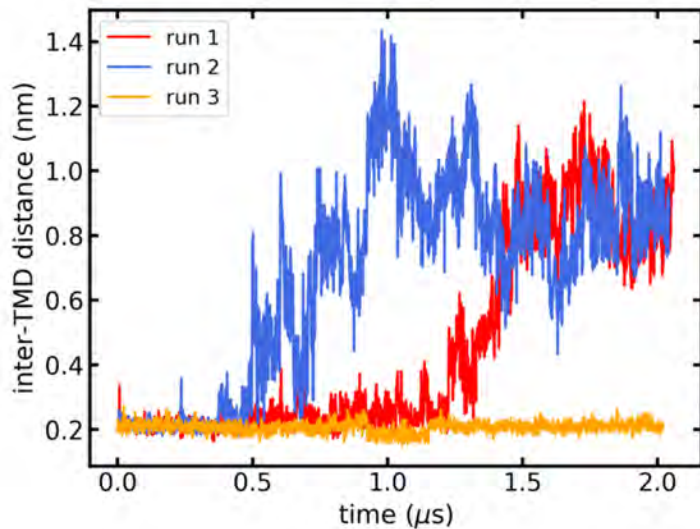
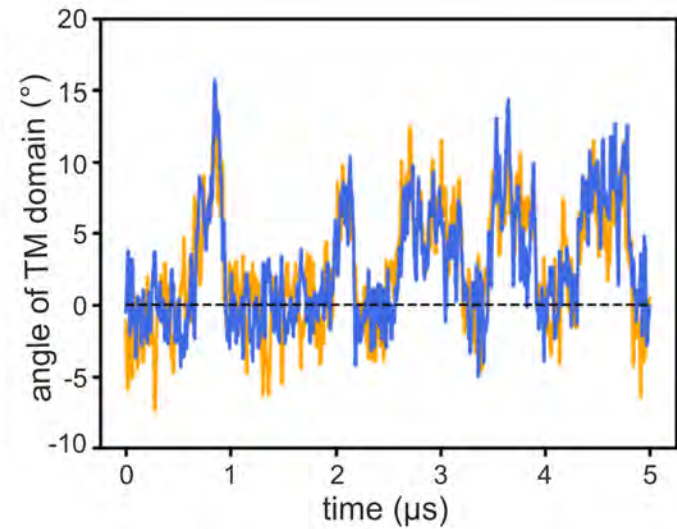
DDM



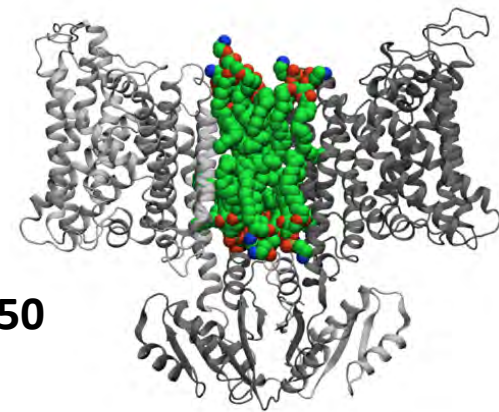
input



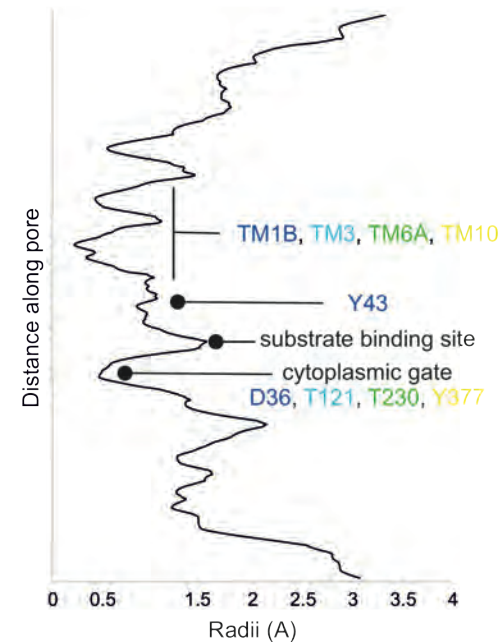
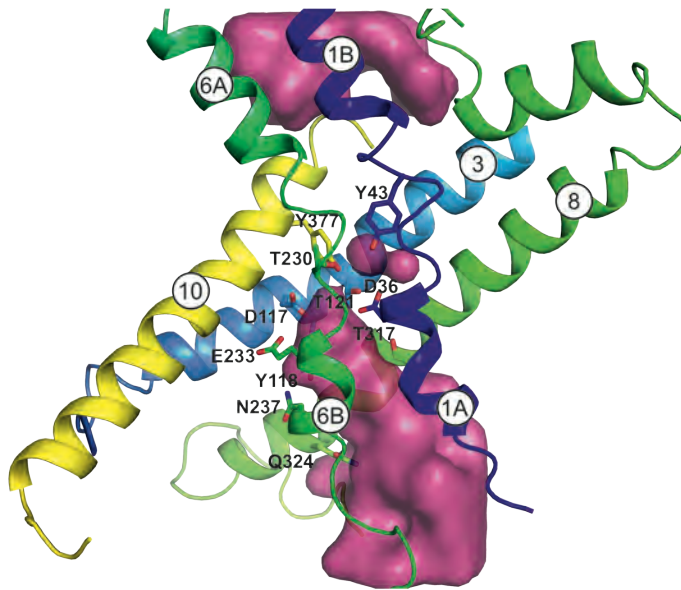
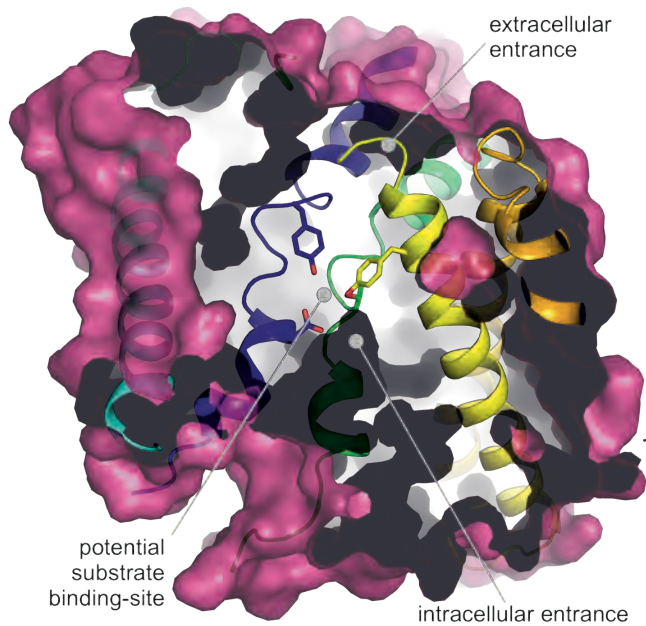
upright dimer



50 : 50



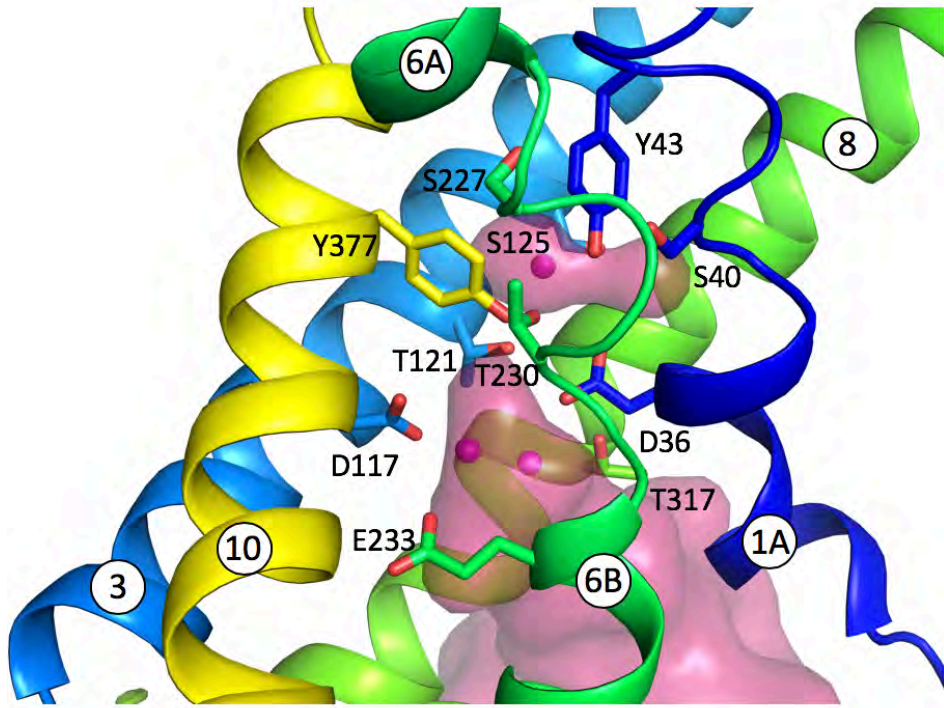
KimA translocation pathway



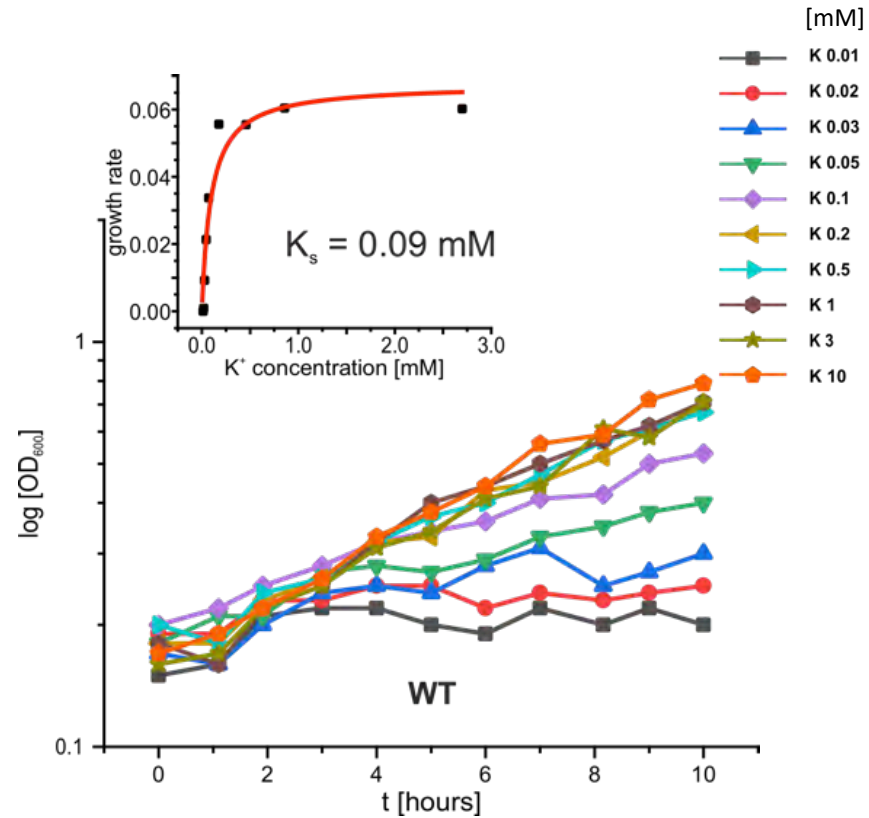
Tascon, Sousa *et al. Nat Commun.* 2020

KimA shows an inward-occluded conformation

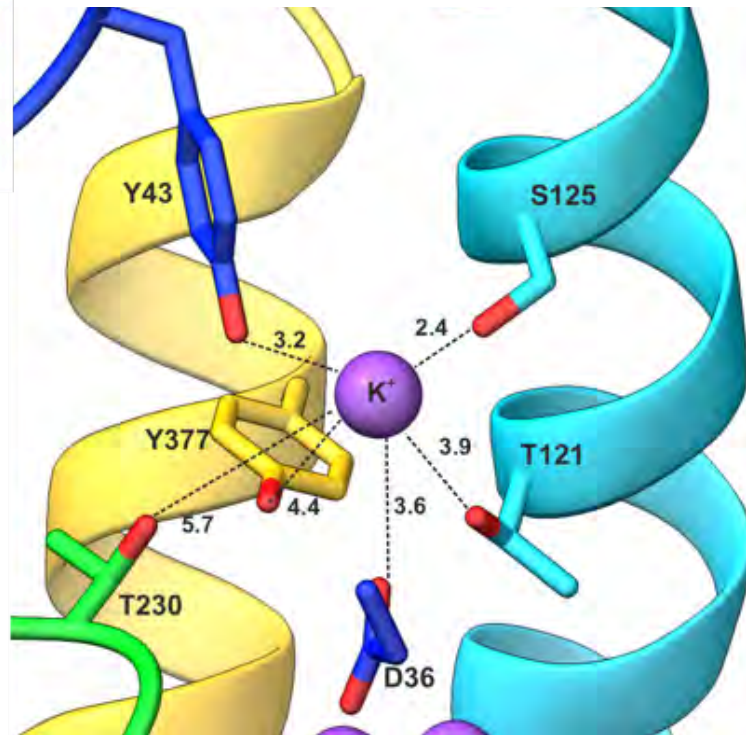
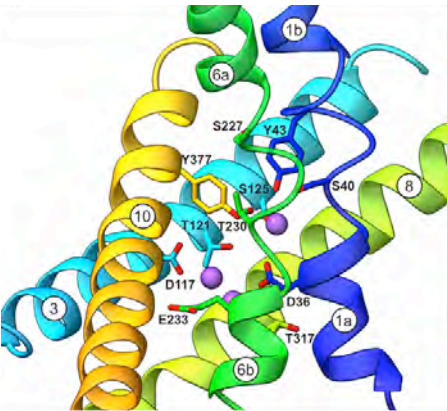
KimA ion binding sites



Tascon, Sousa *et al.* *Nat Commun.* 2020



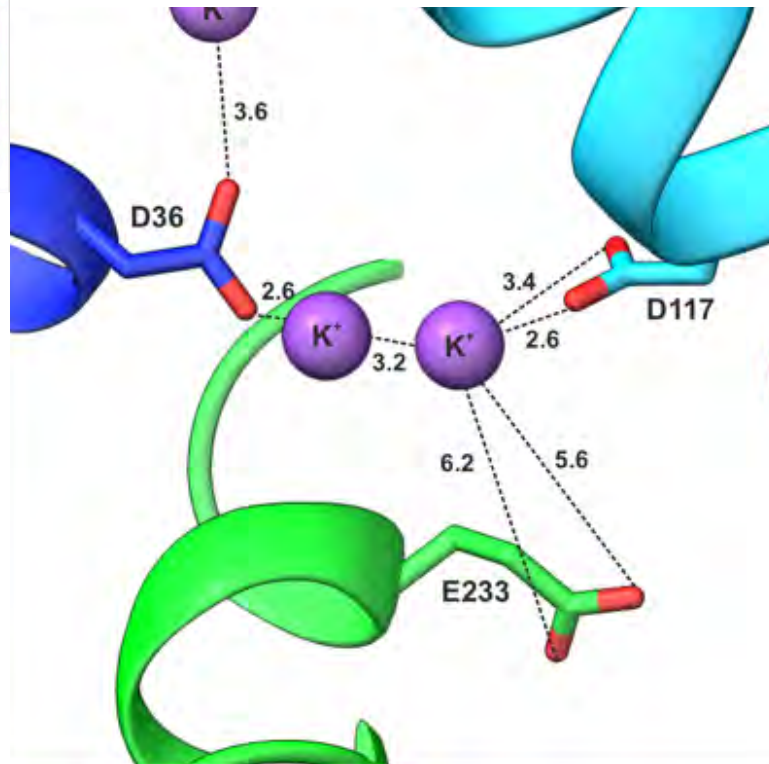
KimA ion binding sites



| Variants | K _s [mM] |
|----------|---------------------|
| WT | 0.09 |
| D36A | n/a |
| D36N | n/a |
| Y43A | n/a |
| T121A | 0.27 |
| S125A | 0.20 |
| T230A | 0.07 |
| Y377F | 11 |

Occluded site represents the K⁺ substrate binding site

KimA ion binding sites



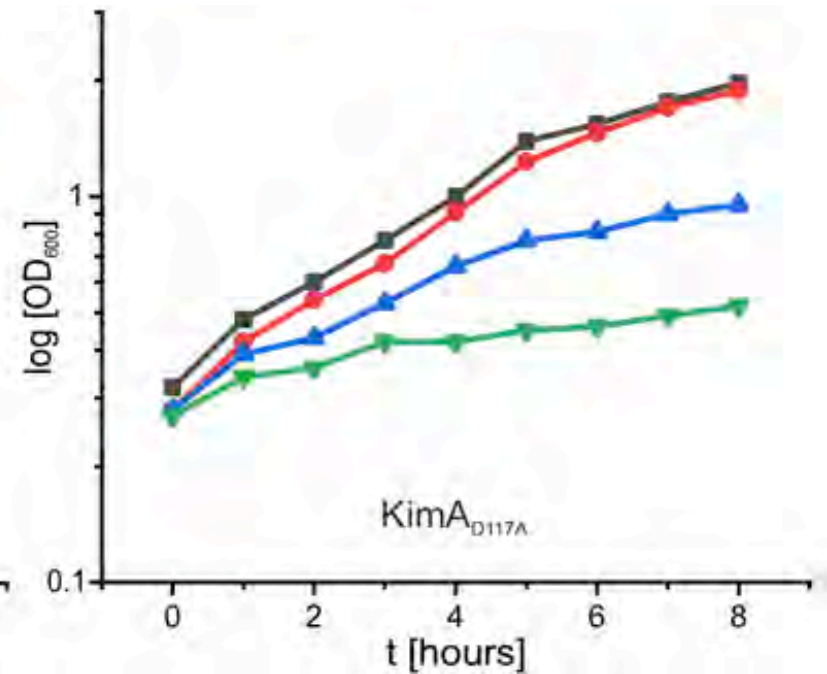
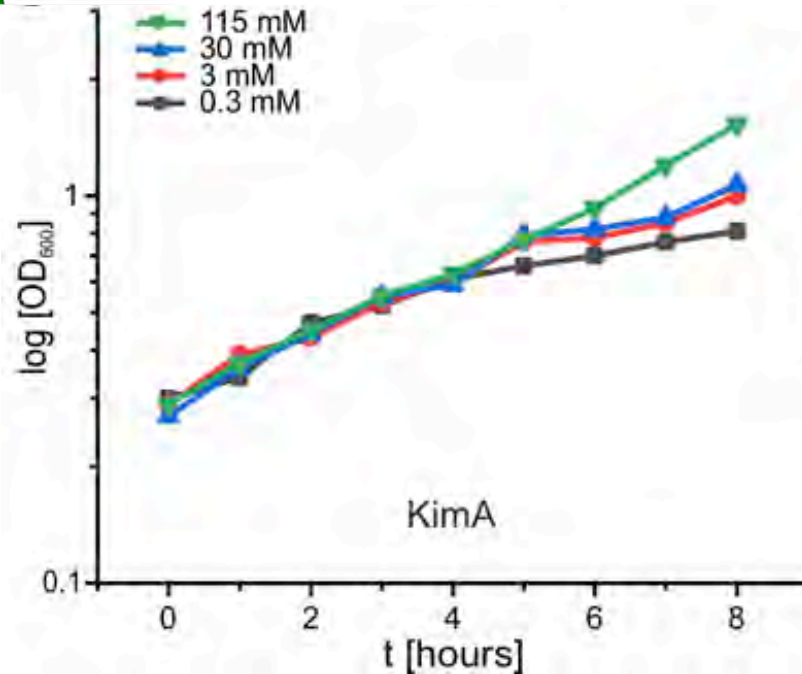
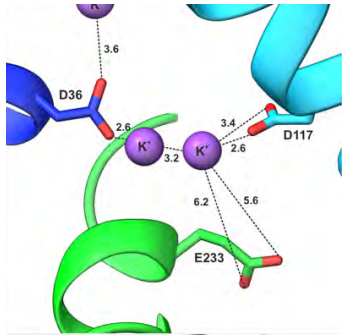
| Variants | K _s [mM] |
|----------|---------------------|
| WT | 0.09 |
| D36A | n/a |
| D36N | n/a |
| D117A | 0.09 |
| D117N | 0.1 |
| D117E | 0.1 |
| E233A | n/a |
| E233Q | n/a |

E233 probably involved in proton coupling

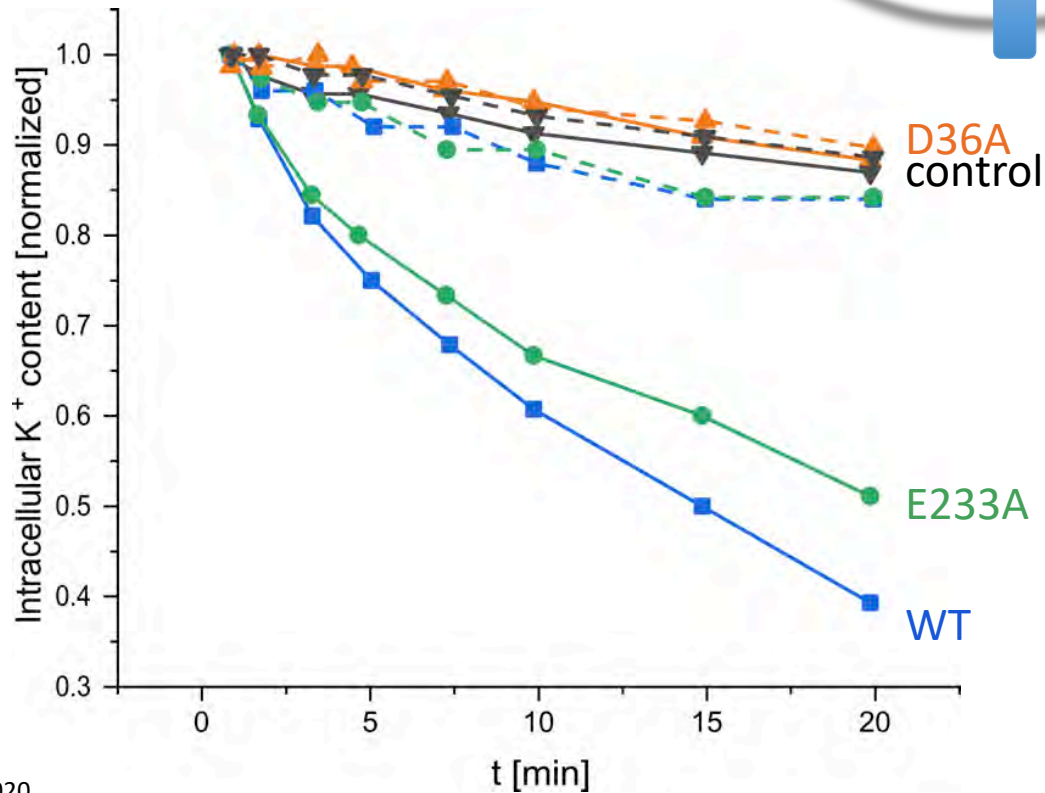
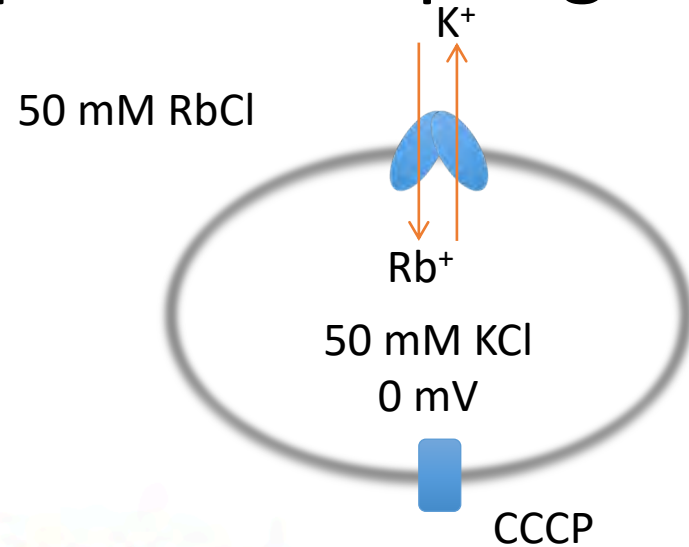
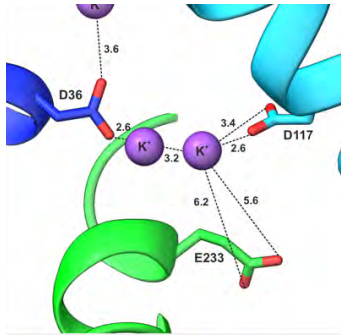
D117 could be involved in regulation: trans-inhibition

D36 plays a central role

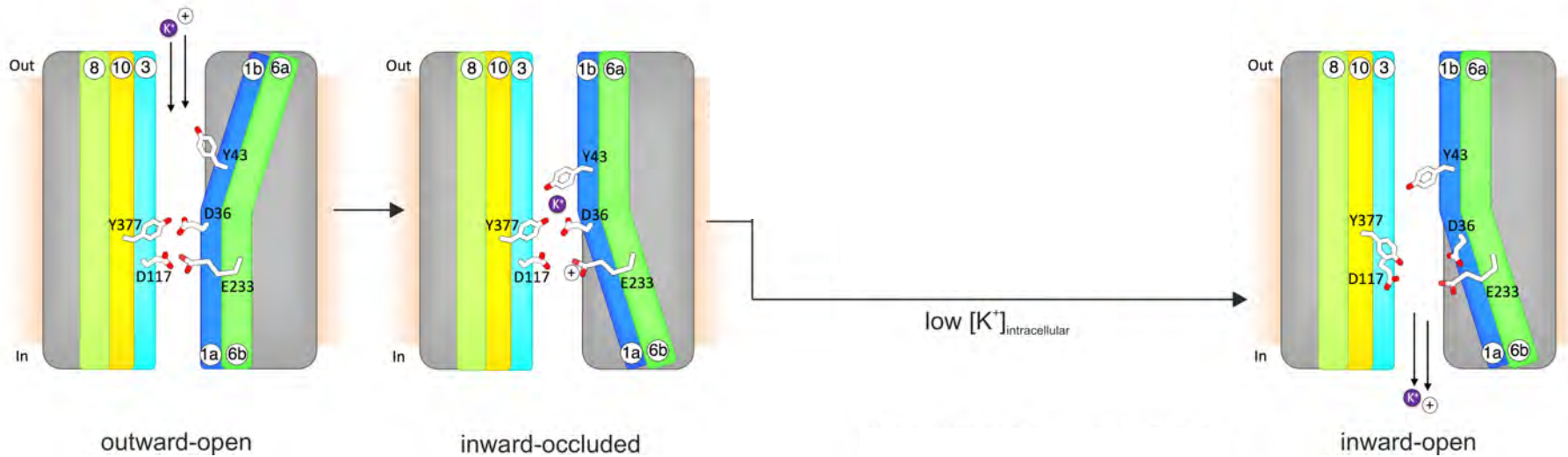
D117 involved in trans-inhibition



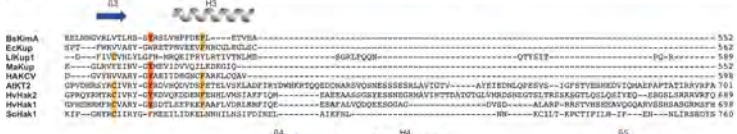
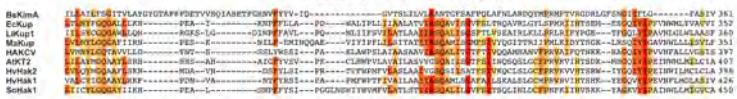
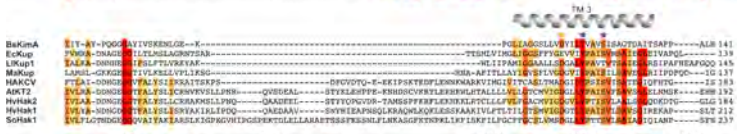
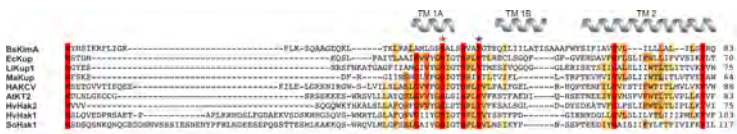
E233 involved in proton coupling



Proton coupled K⁺ transport mechanisms via KimA



KimA is a member of KUP family



| | TM 1A | TM 3 | TM 6B | TM 10 |
|---------------|--------------------|------------------------------|--------------|--------------------|
| BsKimA | LAM LSSD ALSSVAVG' | GLIAGGSLLDVYILTVAVSISAGTDAI | SAITGV EAISN | LIPLYAVGVFIPFTLSQ |
| EcKup | IGVYVGDIGTSPLVT | GLIGGSFFYGVEVITPAISVMSAIEGL | LSITGV EALYA | LAAAYGIAVGTMTVLT |
| LiKup1 | MGIYVGDIGTSPLVT | AMIGGAALLSDGALTPAVTVTSAIEGL | LATTGAEALYS | MEAAAYGLAIVTMTLMT |
| MaKup | MGIYVGDIGTSPIVT | AYIGVSFLVGDGVITPAISILSAVEGL | LCATGGEALYA | LAAAYGLAVTGTMSITG |
| HvHak2 | LGWVFGDLSISPLV | VITCASLTMADGILTPSIVSISATEGL | LAITGV EALYA | IVSAYGFAVSIIVVLLTH |
| HvHak1 | FGVYVGD LSTSPV | VLLGTGMVIGDGLLTPAISVFSAVSGL | CITGAEAMFA | LGNSACLAVMAVMLVTT |
| SoHak1 | LCAYVGDIGTSPLV | VILGTSMVIGDGLTTPAISVLSAVSGL | LCVTGTGEMFA | IGHAYGICVVTTFAITT |
| SoHak1 | LCAYVGDIGTSPLV | CFFGCSLVMSDGLLTPTTSVLSAIAIGI | LSITGTEAMFA | VTAAAYGLGITLDFLVTS |

KimA forms a new subfamily of the KUP family

Acknowledgements

Electron Microscopy:

Joana Sousa

Janet Vonck

Werner Kühlbrandt



MD simulations:

Robin Corey

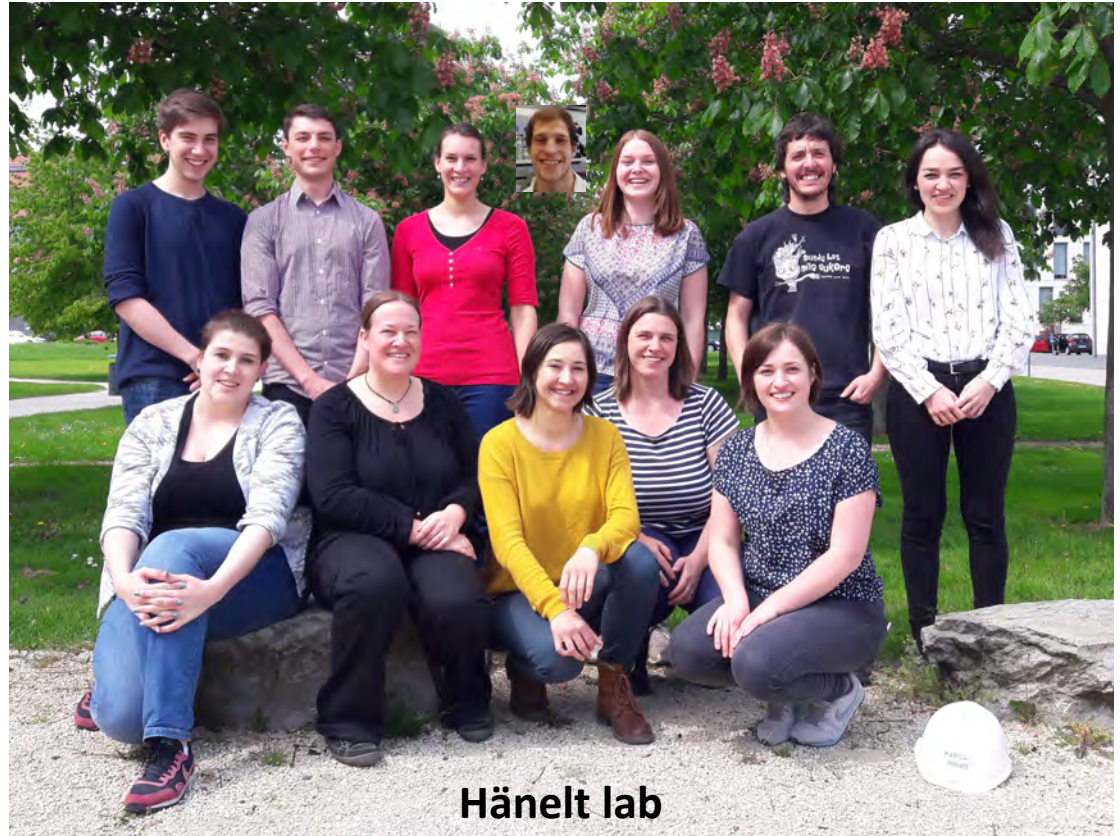
Philip Stansfeld



LILBID:

Oliver Peetz

Nina Morgner



Hänel lab

KimA functional data:

David Griwatz

Nadine Aumüller

Vedrana Mikusevic

SMA provided by:



Thank you!