Chapter 6
Conclusions and future perspectives
Research work in this thesis has explored the physiological linkage between the PtsP-PtsO-PtsN phosphorelay and \( \text{K}^+ \) metabolism in *E. coli*. Studies in this regard have led to the proposal of a model (Fig. 5.24) which postulates a role for dephospho-PtsN in co-ordination of transmembrane \( \text{K}^+ \) fluxes in *E. coli*. The co-ordination is believed to be attained owing to the stimulatory effect of dephospho-PtsN on \( \text{K}^+ \) uptake via the Kdp (Lüttmann et al. 2009, Mörk-Mörkenstein et al. 2017) and the Trk transporters on the one hand and its inhibitory effect on \( \text{K}^+ \) efflux via YcgO on the other. The latter two effects have been proposed based on studies in this thesis. It is believed that the co-ordination exists to allow for attaining a stress responsive \( \text{K}^+ \) limitation in that certain stress(es) may modulate the phosphotransfer capacity of the PtsP-PtsO-PtsN phosphorelay and lead to diminished levels of dephospho-PtsN. The ensuing \( \text{K}^+ \) limitation occurring due to activation of YcgO mediated \( \text{K}^+ \) efflux and attenuation of Trk and Kdp mediated \( \text{K}^+ \) uptake may lead to growth cessation, serving as a survival strategy for stress tolerance.

It is clear from this study that both the inhibitory and the stimulatory effect of dephospho-PtsN on YcgO and the Trk system respectively must operate at the level of activity of the two transporters. As described in chapter 5, the presence of a common structural motif in both TrkA and YcgO, namely the RCK_C domain, a subdomain of the entire RCK domain comprising the N and C-terminal parts (Fig. 5.25), increases the likelihood that dephospho-PtsN interacts with both YcgO and TrkA via this domain. In YcgO this domain is present at its C-terminus and has a predicted cytoplasmic location.

RCK domains are thought to modulate the functions of many \( \text{K}^+ \) transporters of both eukaryotic and prokaryotic origin (Jiang et al. 2001, Roosild et al. 2002), are usually non-integral parts of \( \text{K}^+ \) transporters. In the case of the \( \text{K}^+ \) channels Kch, KefC from *E. coli* and the MthK from *M. thermoautotrophicus* the RCK domain is covalently linked to the channel’s C-terminus and is cytoplasmic. Structural studies on MthK have shown that the 4
primary RCK domains, arising due to tetramerization of the MthK monomers and the 4 extra RCK domains that associate with each of the primary RCK domains constitute an octameric gating ring that modulates calcium and proton mediated channel opening and closing respectively (reviewed in Lingle 2007, Giraldez and Rothberg 2017). RCK domains are also present in the cytoplasmic proteins TrkA and KtrA that oligomerize to form gating rings that associate with the K⁺ transporters TrkH (Cao et al. 2013) and KtrB (Vieira-Pires et al. 2013) respectively. In both the above instances structural studies have revealed the importance of ATP in mediating gating of the ion permeation pathway via its action on the RCK domains (Cao et al. 2013, Vieira-Pires et al. 2013).

Studies in this thesis leave considerable scope for future experimentation. Future studies with respect to the relationship between dephospho-PtsN and the YcgO and Trk K⁺ transporters must be directed towards obtaining mechanistic insights into its modes of action on the two transporters. The notion that dephospho-PtsN is an inhibitor of YcgO activity leads to a prediction, which is that one should be able to isolate mutations in ycgO that lead to a constitutive K⁸, that is K⁺ limited growth in high [K⁺]e medium in an otherwise wild-type strain. It is interesting to note that the V427A substitution that leads to constitutive activation of the KefC K⁺ efflux channel that is negatively regulated by glutathione in E. coli lies in its cytoplasmic RCK domain (Miller et al. 1997). If such substitutions are indeed obtained in the RCK_C domain of YcgO, then it would strengthen the notion that this domain of YcgO is important for YcgO function. Additional experimentation involving determination of the membrane topology of YcgO needs to be performed to show that the RCK_C domain of YcgO is indeed cytoplasmic. The answer to the question “Does dephospho-PtsN interact with YcgO via its C-terminal region?” can only be ascertained by performing protein interaction studies with PtsN, its His-73 derivatives and YcgO. Assuming that the RCK_C domain of YcgO is the locus of interaction with dephospho-PtsN, issues related to how (if at
all) the RCK_C domain of YcgO regulates YcgO activity need to be addressed. A long-term goal would be to reconstitute YcgO mediated K⁺ transport (specifically K⁺/H⁺ antiport) in proteoliposomes and to demonstrate that it is regulated by the phosphorylation status of PtsN.

Whether PtsN interacts with TrkA in a phosphorylation dependent manner and is another subunit of the Trk transporter is another issue that needs to be addressed. Since the defect in Trk mediated K⁺ uptake could be alleviated by overproduction of SapD/F, in the absence of PtsN, it is possible that dephospho-PtsN mediates efficient interaction between SapD/F, the ATP presenting subunits and TrkA, which can be addressed using bacterial two-hybrid analyses and pull-down studies. As in case of YcgO, in principle mutations in trkA can be isolated that are PtsN independent. Their location in TrkA will offer further clues. In addition, whether efficient ATP binding by TrkA requires dephospho-PtsN can be tested. However unlikely, at this point a direct functional interaction between dephospho-PtsN and the K⁺ channel components of the Trk system namely TrkG/TrkH, cannot be ruled out. Lastly K⁺ uptake measurements (flux rates) for the Trk system in presence and absence of PtsN need to be determined.

In studies related to the examination of phosphorylation states of PtsN (in chapter 4), it was intriguing to note, that in the absence of PtsO, both the phosphorylation states of PtsN were detected, regardless of the medium used for growth suggesting that there may be an alternate route of phosphorylation of PtsN in the absence of PtsO, that was PtsP dependent. While evidence presented in chapter 4 appears to exclude Hpr as a likely “PtsO like” candidate in the implied PtsP-Hpr-PtsN phosphotransfer pathway, the effects of absence of Hpr need to be ascertained. Specifically, phosphorylation states of PtsN need to be examined in ptsO, hpr single and double mutants. Lastly additional genetic screens can be set up to identify component(s) of the PtsO independent pathway of phosphotransfer to PtsN.