

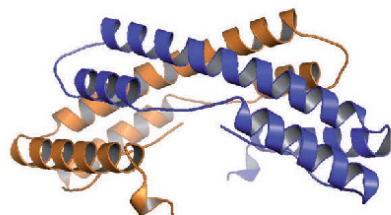


Xiaohui Zhou (Autor)

Structural insight into the Cpx-two-component system

STRUCTURAL INSIGHT INTO THE CPX-TWO- COMPONENT SYSTEM

Xiaohui Zhou



Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

<https://cuvillier.de/de/shop/publications/472>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,
Germany

Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>

Index

Abstract	I
Zusammenfassung	III
Abbreviations	V
Index	VII
1. Introduction	1
1.1. Two-component systems.....	1
1.1.1. Structural insight into the functions of histidine kinases	3
1.1.2. Basic structures and functions of response regulators.....	7
1.1.3. Interaction between histidine kinase and response regulator.....	9
1.1.4. Auxiliary proteins in two-component systems	11
1.2. The Cpx two component system	13
1.3. The third component of the Cpx pathway - CpxP	16
1.4. The goals of my study	17
2. Results.....	19
2.1. Crystallization of the response regulator CpxR	19
2.2. CpxP structure and its functions in Cpx pathway	23
2.2.1. Purification and crystallization of CpxP	23
2.2.2. Secondary structure prediction of CpxP.....	26
2.2.3. Comparison of wild-type CpxP and CpxP Δ 151	28
2.2.4. Crystallization of CpxP Δ 151.....	31
2.2.5. Purification and optimization of Se-Met CpxP Δ 151 Crystals.....	33
2.2.6. X-ray Datasets collection of CpxP Δ 151 and Se-Met CpxP Δ 151 crystals	35
2.2.7. Overall structure of CpxP Δ 151.....	39
2.2.8. Dimer structure of CpxP Δ 151 and its stability	40
2.2.9. CpxP susceptibility for degradation is dependent on its C-terminus	45
2.2.10. Interaction of CpxP with CpxA.....	47
2.2.11. Impact of the C-terminus of CpxP on its function	52
2.2.12. Chaperone-like structure of CpxP.....	54
2.2.13. Crystals of CpxP Δ 151 variants E79R/R144E and R144E	58

3. Discussion	61
3.1. The structure of CpxP	62
3.2. Inhibitory function of CpxP	63
3.3. Quality control function of CpxP	65
3.4. A model for signal integration by CpxP	67
3.5. The Cpx envelope stress pathway: A potential drug target in pathogenic bacteria.....	68
4. Materials and Methods	71
4.1. Bacterial strains, plasmids and primers.....	71
4.2. Media and protocols for cultivation of <i>E. coli</i>	77
4.3. Working with DNA	78
4.3.1. Isolation of plasmid DNA from <i>E. coli</i>	78
4.3.2. Isolation of genomic DNA from <i>E. coli</i>	79
4.3.3. Gel electrophoresis of DNA	79
4.3.4. Extraction of DNA from agarose gels.....	79
4.3.5. Site-Directed mutagenesis.....	79
4.3.6. Amplification of DNA.....	80
4.3.7. Restriction, ligation and sequencing of DNA.....	80
4.3.8. Preparation of competent cells and transformation	81
4.4. Protein purification and production.....	83
4.4.1. Expression of proteins and preparation of cytosolic fraction.....	83
4.4.2. Purification of responds regulator CpxR	83
4.4.3. Purification of histidine kinase CpxA sensor domain	84
4.4.4. Purification of CpxP, CpxP Δ 151 and its variants	85
4.5. Protein analysis manipulation.....	86
4.5.1. Analysis of protein concentration.....	86
4.5.2. SDS-PAGE and Coomassie staining	86
4.5.3. Limited proteolysis of proteins	88
4.5.4. CD spectra.....	88
4.5.5. Western blotting.....	89
4.5.6. β -galactosidase activity analysis.....	90

4.6. Biochemistry Methods	91
4.6.1. Cell fractionations and determination of CpxP expression	91
4.6.2. Size exclusion chromatography (SEC)	91
4.6.3. Protein crystallization	92
4.6.4. Data collection and structure determination	93
4.7. Antibody preparation of CpxP and CpxR	94
5. References.....	95
Acknowledgement.....	111