

Appendix B - Lists and directories

List of abbreviations

| | |
|-----------------------------------|--|
| AEC | anion exchange chromatography |
| APBS | adaptive Poisson-Boltzmann solver |
| <i>Bc</i> -Csp | <i>Bacillus caldolyticus</i> Csp |
| <i>Bs</i> -CspB | <i>Bacillus subtilis</i> Csp |
| calc. | calculated |
| CD | circular dichroism |
| Csp / CSP | a specific cold shock protein / cold shock protein(s) (in general) |
| CSD | cold shock domain(s) |
| DEAE sepharose | diethyl-amino-ethyl sepharose (a column material for anion exchange chromatography) |
| diglycerides | diacylphosphatidylglycerides |
| dT ₆ , dC ₆ | deoxyribo hexathymidine, deoxyribo hexacytidine |
| dT ₇ , dC ₇ | deoxyribo heptathymidine, deoxyribo heptacytidine |
| DNA | deoxyribonucleic acid |
| dNTP | deoxyribonucleoside triphosphate |
| <i>Ec</i> -CspA | <i>Escherichia coli</i> CspA |
| EDTA | ethylene-diamine-tetraacetate |
| FPLC | fast phase liquid chromatography |
| GFC | gel filtration chromatography |
| HCOH | formaldehyde |
| HIC | hydrophobic interaction chromatography |
| IPTG | isopropyl thiogalactoside |
| K_A , K_D | equilibrium association and dissociation constants (see Equation 2.6 & Equation 2.7) |
| k_{on} , k_{off} | association and dissociation rate constants |
| k_{obs} | observed quasi first-order rate constant in stopped-flow analyses (see Equation 2.10) |
| LB | Luria-Bertani medium for bacterial cell culture |
| mRNA | messenger RNA |
| mol. | molecular |
| MPD | 2-methyl-2,4-pentanediol |
| MR | molecular replacement |
| OB-fold | oligonucleotide / oligosaccharide binding fold |
| PCR | polymerase chain reaction |
| pH | <i>potentia hydrogenii</i> ($\log c_{H_3O^+}$), measure for acidity in aqueous solutions |
| pI | isoelectric point (pH at which a molecule has a net charge of 0) |
| PDB | Protein Data Bank, see [111] |

Appendix B

| | |
|----------------|--|
| RMSD | root mean square deviation |
| RNA | ribonucleic acid |
| RNP | ribonucleoprotein |
| rpm | rotations per minute |
| rxn. | reaction |
| PAGE | poly-acrylamide gel electrophoresis |
| PEG | polyethylene glycol |
| SDS | sodium dodecyl sulfate |
| ssDNA | single-stranded DNA |
| ssRNA | single-stranded RNA |
| TBE | TRIS-borate-EDTA buffer |
| TCA | tri chlorine acetate |
| TEMED | N,N,N',N'-tetramethylethylenediamine |
| TLS | translational, librational, & screw [parameters] |
| T_M | melting temperature |
| <i>Tm</i> -Csp | <i>Thermotoga maritima</i> Csp |
| TRIS | tris(hydroxymethyl)-aminomethan |
| UV / vis | ultraviolet / visible (light) |
| vs. | <i>versus</i> (= in comparison to) |

physical units

| | |
|-----|---|
| °C | temperature unit, degrees Celsius |
| Å | distance unit, Ångstroem (10^{-10} m) |
| A | electrical currency unit, Ampère |
| Da | molecular weight unit, Dalton, ~ g / mol |
| g | mass unit, gram |
| h | time unit, equal 60 minutes |
| l | volume unit, liter |
| m | distance unit, meter |
| mol | molecular unit, mole, equals $6.023 \cdot 10^{23}$ particles of a substance |
| M | concentration unit, molar, moles per liter |
| min | time unit, minute, equals 60 seconds |
| psi | pressure unit, 1 psi = 6894.8 Pa |
| Pa | pressure unit, Pascal (pressure unit) |
| s | time unit, second |
| u | (biological) activity unit, describes the activity of an enzyme |
| V | electrical voltage unit, Volt |

prefixes defining orders of magnitude

| | | | |
|---|------------------|---|--------------|
| p | pico, 10^{-12} | k | kilo, 10^3 |
| n | nano, 10^{-9} | M | mega, 10^6 |
| μ | micro, 10^{-6} | G | giga, 10^9 |
| m | milli, 10^{-3} | | |

Directory of figures

| Figure | Title | Page |
|---------------|---|-------------|
| 1.1 | Folding can be multi-state. | 8 |
| 1.2 | Energy diagram of a protein, which folds according to a two-state mechanism. | 8 |
| 1.3 | Temperature denaturation curves for histidines of ribonuclease A. | 9 |
| 1.4 | Nucleic-acid building blocks and Watson-Crick basepairing | 11 |
| 1.5 | Two conformations of the DNA double helix. | 12 |
| 1.6 | Cold-inducible genes of <i>Bacillus subtilis</i> and <i>Escherichia coli</i> . | 15 |
| 1.7 | Model for the function of cold shock proteins as RNA chaperones. | 19 |
| 2.1 | Anion-exchange chromatography of protein variant <i>Bs</i> -CspB R56A | 32 |
| 2.2 | Hydrophobic interaction chromatography of <i>Bs</i> -CspB R56A. | 33 |
| 2.3 | Gel-filtration chromatography of <i>Bs</i> -CspB R56A. | 34 |
| 2.4 | UV / vis spectra of single components and purified CSP·dT ₆ complexes. | 35 |
| 2.5 | Principles of analytical equilibrium ultracentrifugation. | 44 |
| 2.7 | Mass determination of the <i>Bs</i> -Csp·dT ₆ complex in solution. | 44 |
| 2.8 | Phase diagram of protein crystallization. | 45 |
| 2.9 | Protein crystallization setups using the hanging and the sitting drop method. | 46 |
| 2.10 | Bragg's law visualized schematically. | 48 |
| 3.1 | Protein crystallization of the <i>Bs</i> -CspB·dT ₆ complex. | 63 |
| 3.2 | X-ray diffraction data collection of a <i>Bs</i> -CspB·dT ₆ complex crystal. | 64 |
| 3.3 | Refinement progress of a <i>Bs</i> -CspB·dT ₆ complex crystal structure. | 65 |
| 3.4 | Unit cell content of the <i>Bs</i> -CspB·dT ₆ and <i>Bc</i> -Csp·dT ₆ crystal structures. | 66 |
| 3.5 | Superposition of ligand-free and dT ₆ -complexed CSP. | 66 |
| 3.6 | Trp8 forms a hydrophobic contact with its equivalent from a symmetry-related molecule in the <i>Bs</i> -CspB·dT ₆ structure. | 67 |
| 3.7 | The ligand nucleobases interact with an extended hydrophobic platform and surrounding polar groups on the CSP surface. | 69 |
| 3.8 | DNA single strands adopt an irregular conformation upon binding to CSP | 71 |
| 3.9 | A continuous arrangement is formed by <i>Bs</i> -CspB and dT ₆ molecules. | 71 |
| 3.10 | Melting curves of <i>Bs</i> -CspB mutants determined by CD spectroscopy. | 73 |
| 3.11 | Determination of kinetic rates (k_{on} , k_{off}) describing dissociation and association of <i>Bs</i> -CspB·oligonucleotide complexes by stopped-flow. | 75 |
| 3.12 | Determination of equilibrium dissociation constants (K_D) of <i>Bs</i> -CspB·oligonucleotide complexes using fluorescence titrations. | 75 |
| 3.13 | Temperature dependence on preferential binding of heptapyrimidines by CSP. | 79 |

| Figure | Title | Page |
|---------------|--|-------------|
| 3.14 | Nucleobase headgroups interact with protein groups from subsite 2 through hydrogen bonds reminiscent of Watson-Crick basepairs. | 81 |
| 3.15 | Intermolecular interactions between CSP and hexathymidine. | 82 |
| 3.16 | Schematic overview of CSP·oligonucleotide interactions. | 84 |
| 3.17 | Two examples of predicted terminator sites which contain heptanucleotide sequences in agreement with presumed binding preferences of CspB | 85 |
| 3.18 | Superimposition of <i>Bacillus</i> CSP crystal structures from the PDB. | 89 |
| 3.19 | The binding site for nucleic acids is conserved throughout the CSP and Y-box proteins. | 89 |
| 4.1 | Protein crystallization of the <i>Bc</i> -Csp·dT ₆ complex. | 93 |
| 4.2 | X-ray diffraction data collection of a crystal featuring the <i>Bc</i> -Csp dT ₆ complex. | 94 |
| 4.3 | Refinement progress of the <i>Bc</i> -Csp·dT ₆ complex crystal structure. | 94 |
| 4.5 | Initial and optimized molecular replacement solutions for <i>Bc</i> -Csp·dT ₆ in space group P2 ₁ . | 96 |
| 4.4 | Formation of domain-swapped tetramers in the <i>Bc</i> -Csp·dT ₆ crystal. | 96 |
| 4.6 | Symmetry elements parallel to <i>b</i> and <i>c</i> axes indicate a higher-symmetry space group than P2 ₁ . | 97 |
| 4.7 | Zero-layer and first-layer precession images calculated from diffraction data from a <i>Bc</i> -Csp·dT ₆ dataset processed in P1. | 98 |
| 4.8 | Molecular replacement solutions for <i>Bc</i> -Csp·dT ₆ in the space group P2 ₁ 2 ₁ 2. | 99 |
| 4.9 | Region of the domain swap in the <i>Bc</i> -Csp·dT ₆ structure revealed by its difference electron density. | 100 |
| 4.10 | Comparison of open (domain-swapped) and closed states of <i>Bc</i> -Csp. | 102 |
| 4.11 | Topology plot of the <i>Bc</i> -Csp architecture. | 103 |
| 5.1 | Crystallization of the <i>Bs</i> -CspB variant M1R/E3K/K65I. | 107 |
| 5.2 | Crystallization of the <i>Bs</i> -CspB variant A46K/S48R. | 108 |
| 5.3 | Diffraction images of stabilized <i>Bs</i> -CspB variants. | 109 |
| 5.4 | Refinement progress of stabilized <i>Bs</i> -CspB variants. | 109 |
| 5.5 | Electrostatic surface potential of <i>Bs</i> -CspB wildtype and mutants. | 113 |
| 5.6 | Stabilizing effects associated with individual amino acid changes in <i>Bs</i> -CspB variant M1R/E3K/K65I. | 114 |
| 5.7 | Stabilizing effects associated with individual amino acid changes in <i>Bs</i> -CspB variant A46K/S48R. | 117 |
| 5.8 | Frequency plot based on an alignment of 250 CSP sequences. | 119 |

Directory of tables

| Table | Title | Page |
|-------|---|------|
| 2.1 | A short protocol for silver staining of polyacrylamide gels. | 28 |
| 2.2 | A protocol for site-directed mutagenesis of bacterial plasmids. | 30 |
| 2.3 | Primers used for the generation of mutant <i>Bs</i> -CspB variants by site-directed mutagenesis. | 30 |
| 2.4 | Molar extinction coefficients of proteins. | 37 |
| 2.5 | Molar extinction coefficients of oligonucleotides. | 38 |
| 3.1 | Building, refinement and evaluation of the atomic model based on diffraction data from <i>Bs</i> -CspB·dT ₆ . | 65 |
| 3.2 | Equilibrium dissociation constants (K_D) of <i>Bs</i> -CspB and mutant variants in complex with dT ₇ . | 74 |
| 3.3 | Equilibrium dissociation constants (K_D) and kinetic association (k_{on}) and dissociation (k_{off}) rate constants of <i>Bs</i> -CspB·heptapyrimidine complexes. | 76 |
| 3.4 | Dissociation constants (K_D) of <i>Bc</i> -Csp·heptapyrimidine complexes. | 76 |
| 3.5 | Binding preferences of <i>Bs</i> -CspB and <i>Bc</i> -Csp for heptanucleotides containing thymine and cytosine at individual positions. | 77 |
| 3.6 | Binding preferences of <i>Bs</i> -CspB for heptanucleotides containing pyrimidines and purines at individual positions. | 78 |
| 4.1 | Building, refinement and evaluation of the atomic model based on diffraction data from <i>Bc</i> -Csp·dT ₆ . | 95 |
| 5.1 | Data collection of <i>Bs</i> -CspB M1R/E3K/K65I and <i>Bs</i> -CspB A46K/S48R. | 108 |
| 5.2 | Building, refinement and evaluation of the atomic models of <i>Bs</i> -CspB variants M1R/E3K/K65I and A46K/S48R | 110 |
| 5.3 | Stability data for variants of <i>Bs</i> -CspB. | 112 |

Directory of equations

| Equation | Title | Page |
|----------|--|------|
| 1.1 | The Gibbs-Helmholtz equation - definition of the Gibbs free energy. | 14 |
| 1.2 | The Arrhenius equation - definition of reaction rate constants (k). | 14 |
| 2.1 | Lambert-Beer's law - dependence of UV / vis absorbance on concentration. | 37 |
| 2.2 | Definition of difference in circular polarized absorbance ΔA . | 38 |
| 2.3 | Dependence of ΔA on concentration. | 38 |
| 2.4 | Definition of the molar dichroism $\Delta \epsilon_\lambda$ | 38 |
| 2.5 | Definition of the molar ellipticity θ . | 38 |
| 2.6 | Definition of the equilibrium dissociation constant (K_D). | 39 |
| 2.7 | Definition of the equilibrium association constant (K_A). | 39 |

| Equation | Title | Page |
|----------|--|------|
| 2.8 | Definition of the quench (Q) in fluorescence titration experiments. | 40 |
| 2.9 | Calculation of K_D by nonlinear fitting of data from fluorescence titrations. | 41 |
| 2.10 | Definition of a quasi first-order rate constant describing pre-equilibrium complex formation by stopped-flow analyses. | 42 |
| 2.11 | Determination of association (k_{on}) and dissociation (k_{off}) rate constants by nonlinear fitting of data from stopped-flow analyses. | 42 |
| 2.12 | Calculation of dissociation rate constants (k_{off}) using K_D and k_{on} | 42 |
| 2.13 | Determination of molecular masses from absorbance data by analytical ultracentrifugation. | 43 |
| 2.14 | Dependence of the exponential coefficient F on biophysical properties of system and molecular sample in analytical ultracentrifugation. | 43 |
| 2.15 | Bragg's law – dependence of positive interference of diffracted X-rays on wavelength, distance and difference in pathlength. | 49 |
| 2.16 | Definition the phase shift of a diffracted X-ray beam caused by a single atom. | 49 |
| 2.17 | Definition of the structure factor $\vec{F}(h, k, l)$ based on diffraction data. | 50 |
| 2.18 | Definition of the structure factor $\vec{F}(h, k, l)$ based on atomic models. | 50 |
| 2.19 | Correlation of structure-factor amplitudes based on diffraction intensities | 50 |
| 2.20 | Definition of R_{sym} , a factor that describes the accuracy of diffraction data by comparing intensities of equivalent reflections. | 51 |
| 2.21 | The Patterson function – a function based on structure factor amplitudes which reveals interatomic distance vectors in a crystal. | 52 |
| 2.22 | A scoring function involving Patterson functions in the search for orientational parameters of a structural model in molecular replacement. | 53 |
| 2.23 | Calculation of electron density maps from structure factors by Fourier transformation. | 54 |
| 2.24 | Calculation of structure factors from electron density maps by performing a Fourier backtransform. | 54 |
| 2.25 | Calculation of a $2F_o - F_c$ difference electron density map. | 54 |
| 2.26 | Calculation of a $F_o - F_c$ difference electron density map. | 54 |
| 2.27 | Definition of crystallographic R -factors, which describe levels of agreement between structural models and diffraction data. | 55 |
| 2.28 | Principles of restraint refinement involving stereochemical parameters and X-ray diffraction data. | 56 |
| 2.29 | Introduction of the Debye-Waller factor, a measure of atomic displacement, into the structure factor equation. | 56 |
| 2.30 | Determination of the radius of displacement from a Debye-Waller factor. | 56 |

