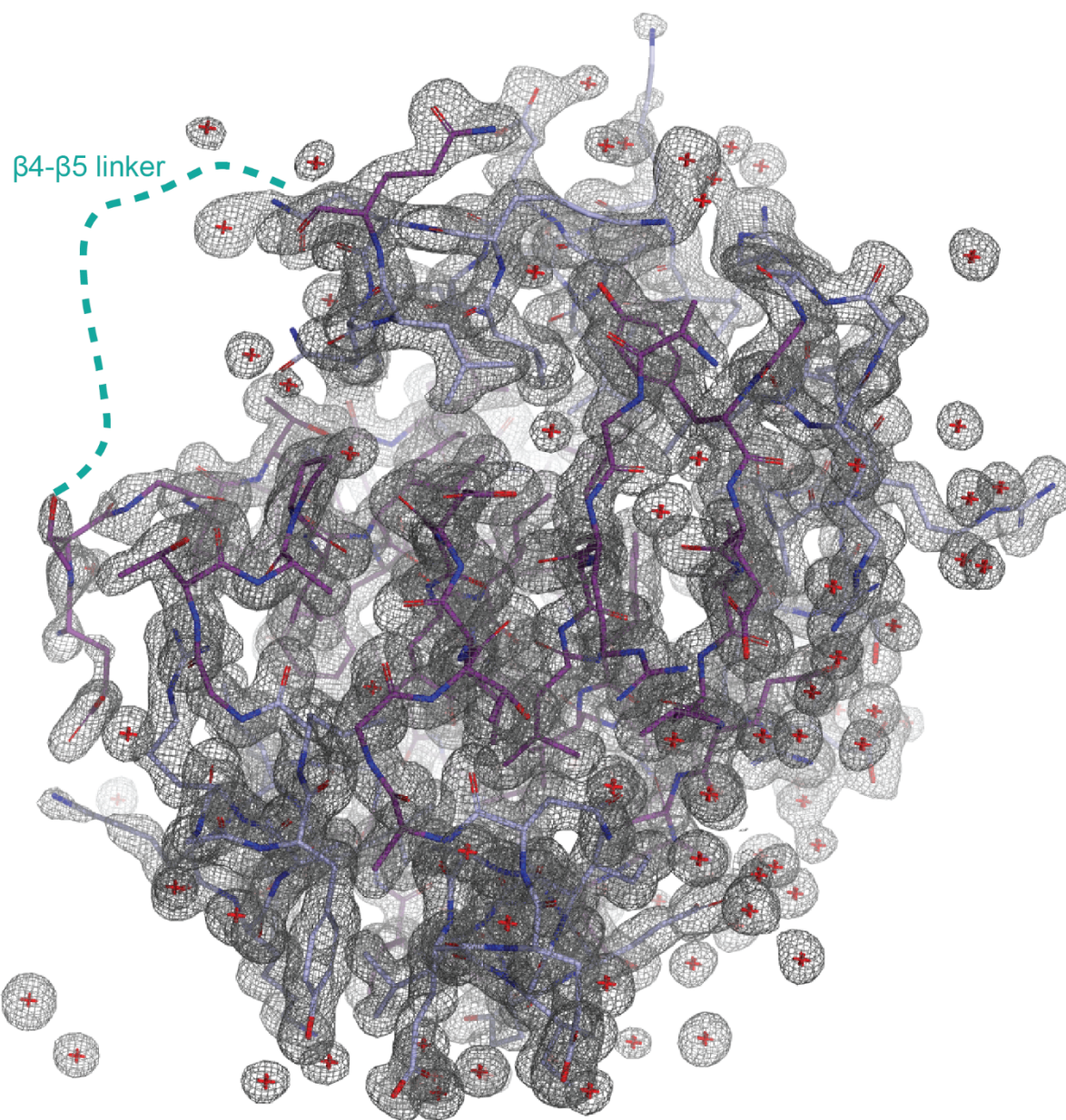
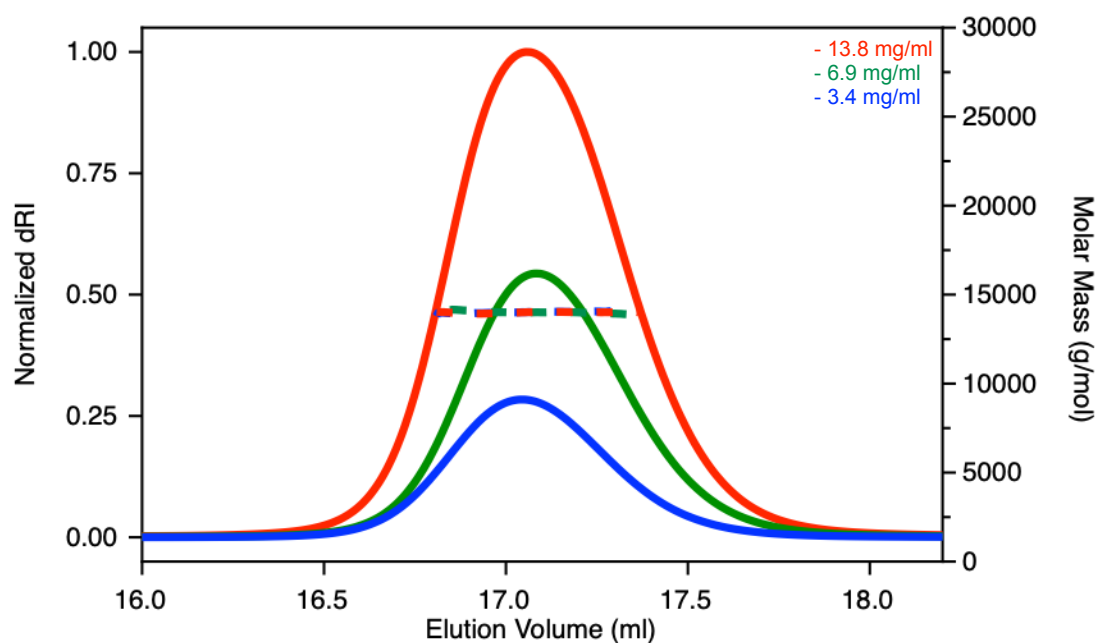


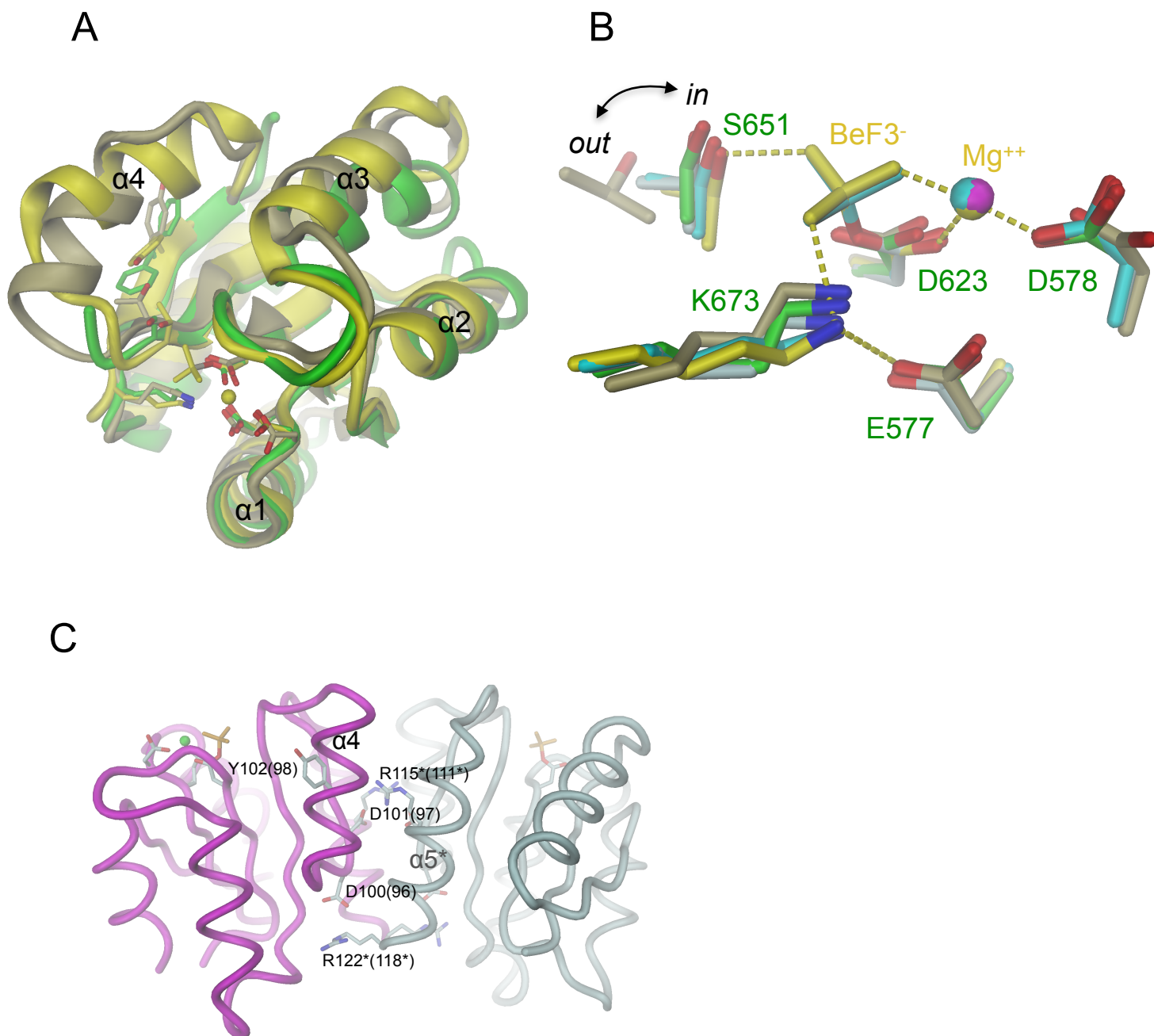
Supplementary Figures to Brüderlin et al., Intermediaries in phosphotransfer: structural features distinguishing Rec domains of hybrid histidine kinases from response regulator homologs



Supplementary Figure S1. Electron density and full model of CckA^{Rec}. The electron density is clearly defined for the entire molecule with the exception of the $\beta 4 - \beta 5$ linker. Water molecules are shown as red crosses.



Supplementary Figure S2. SEC-MALS profile of CckA^{Rec}. Molecular mass values (right axis) are represented by dotted lines and change in refractive index (left axis) by solid lines. Protein concentrations are shown at the top right of the figure.



Supplementary Figure S3. Structure comparison of CckA^{Rec} with native and activated Rec domains.

(A) Superposition of CckA^{Rec} (green), native PhoB (1b00, khaki), and BeF₃⁻ modified PhoB (1zes, yellow). Residues of the active site and the Y/F residue of the switch are shown in full. Note that F670 of CckA^{Rec} exhibits two alternative conformations that coincide with the outward and inward orientation seen in native and activated PhoB, respectively. For the superposition, only the Cα - positions of the depicted residues except Y/F were used.

(B) Close-up of active sites shown in (A) together with those of native (6is2, white) and BeF₃⁻ modified ArlR (6is1, cyan). Note that all structures, including that of CckA^{Rec} (green) exhibit the same arrangement (activated conformation, rmsd = 0.40 - 0.58 Å for all atoms) with the exception of native PhoB (khaki), which has the S/T residue in an "out" position (rmsd = 0.77 Å).

(C) Dimeric structure of BeF₃⁻ modified PhoB (1zes) with selected residues, including conserved charged residues of the interface, in full. Asterisks indicate symmetry related residues. CtrA residue numbers are given in brackets.

A

CckA^{Rec}

CpdR



MrrA

CtrA^{Rec}ShkA^{Rec}

B

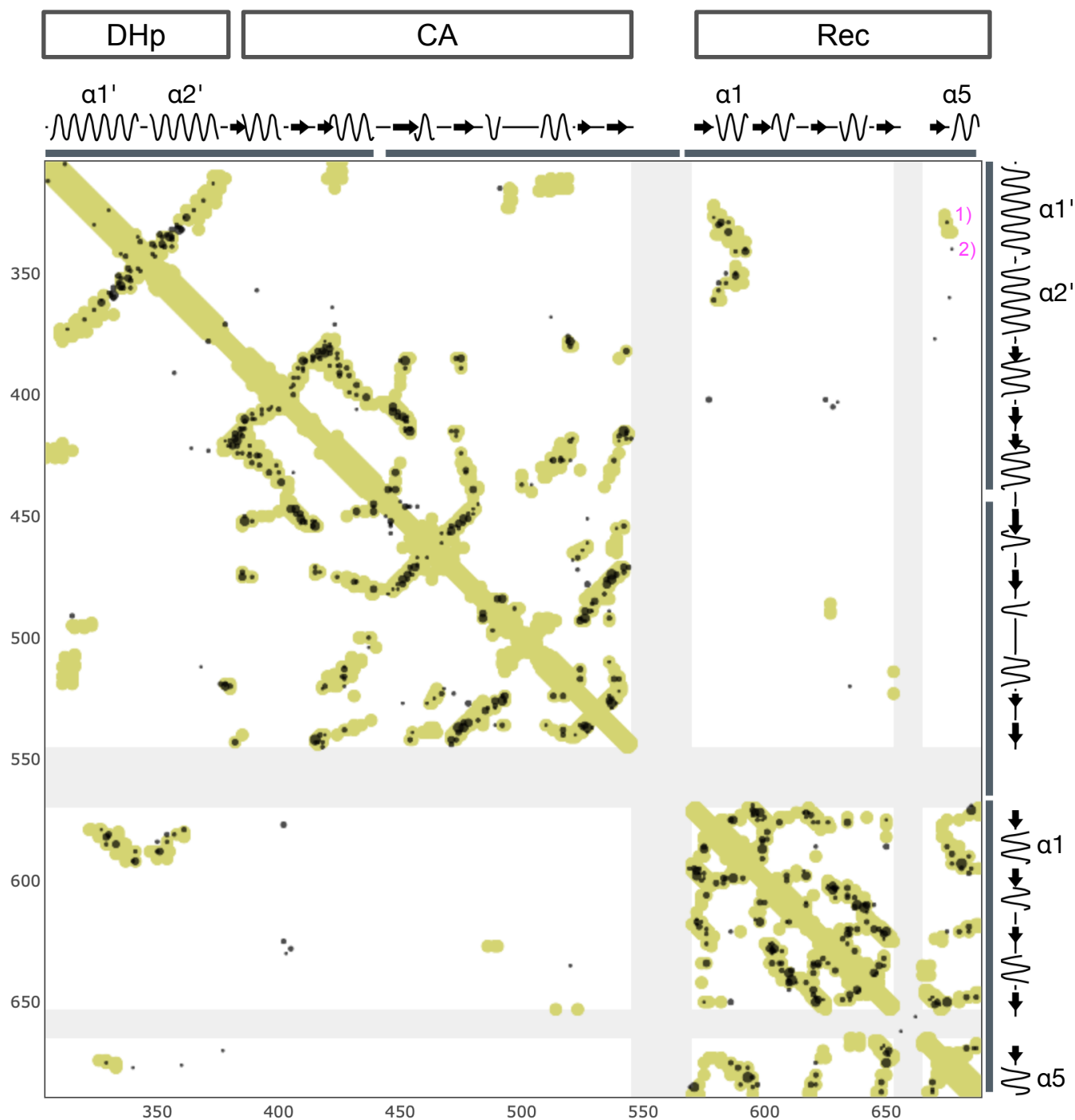
log(E) score of sequence/motif match

organism	protein	UNIPROT	Rec domain			HMM Rec motif				
			type	PDB	FATGUY motif	CckA ^{Rec}	CpdR	MrrA	CtrA ^{Rec}	ShkA ^{Rec}
<i>C. cresc</i>	CckA	Q9X688	HHK	6tne	FISGYAE	-43	-33	-11	-16	-16
<i>C. cresc</i>	CpdR	Q9AA62	single		FITGFAA	-32	-49	-12	-20	-22
<i>C. cresc</i>	MrrA	Q9A428	single		FASGYGE	-14	-14	-49	-9	-10
<i>C. cresc</i>	CtrA	P0CAW8	RR			-19	-24	-13	-51	-24
<i>C. cresc</i>	ShkA	Q9ABT2	HHK *)	6qrj		-14	-20	-12	-27	-52
<i>B. subtilis</i>	Spo0F	P06628	single	2ftk	IMTAYGE	-26	-31	-17	-24	-24
<i>R. meliloti</i>	Sma011	Q930Y6	single	2lpm	FATGYGS	-15	-16	-47	-13	-11
<i>S. melonis</i>	SdrG	A0A0D1M	single	5ieb	FATGGS	-10	-12	-44	-8	-6
<i>P. aerug</i>	PA1611	Q9I3B1	HHK	7c1j		-16	-22	-14	-29	-54
<i>S. cerevisiae</i>	SLN1	P39928	HHK	2r25		-14	-16	-6	-16	-37
<i>A. thaliana</i>	AHK5	Q3S4A7	HHK	4euk		-14	-18	-9	-17	-43
<i>A. thaliana</i>	CKI1	Q22267	HHK	3mmn		-12	-12	-6	-13	-30
<i>E. coli</i>	RcsC	P0DMC5	HHK *)	2ayz		-22	-26	-11	-22	-35

Supplementary Figure S5. Sequence logos for five distinct Rec groups and correlation (E-values) of selected sequences against derived HMM motifs.

(A) Rec logos as derived from the alignment of homologous sequences retrieved by HMMER. The names of the founder sequences are indicated. For details see Material and Methods.

(B) HMMSCAN E-scores of full-length sequences against the HMM Rec motifs corresponding to the logos shown in (A). Orange background: $E < 1e-40$, dark grey: $E < 1e-30$, light grey: $E < 1e-25$. *): C-terminal domain of the two Rec domains.



Supplementary Figure S6. HHK co-variation analysis. EVcoupling matrix based on 8067 sequences retrieved with CckA query sequence and an E-value of $1e-60$. All peaks (scaled according to score) with a probability $>95\%$ are shown. Yellow dots indicate contacts with a distance $< 6\text{\AA}$ in the CA_DH_p_Rec phosphotransfer model of CckA (Fig.5). Some helices of the DH_p domain ($\alpha 1'$, $\alpha 2'$) and the Rec domain ($\alpha 1$ to $\alpha 2$) are labeled on the axes. Significant Rec / DH_p (top right part of the matrix) covariation values are found almost exclusively between Rec- $\alpha 1$ and the $\alpha 1'$, $\alpha 2'$ DH_p helices (see the zoom-in of Fig. 9). The peaks marked with 1) and 2) correspond to T329/I675 and H340/I677 (distance $10\text{\AA}$), respectively, and involve Rec $\beta 5$ - $\alpha 5$. All other inter-domain correlations are probably spurious or indirect, since all their distances are $> 20\text{\AA}$.