# Supplementary Information

# Acetylation and phosphorylation control both local and global stability of the chloroplast $F_1$ ATP synthase

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## Tables

**Table S1: PTMs identified in the cATPase.** The protein subunit, the modified residue, the type of PTM, the identified peptide sequence and the highest observed Mascot and MaxQuant score are given. It is indicated if the respective PTMs were identified before. Note that some modified sites could not unambiguously be assigned.

| Subunit | Site | PTM | Peptide sequence | max Mascot score | max MaxQuant score | identified previously |
|---|---|---|---|---|---|---|
| α | N-Term | acetylation | *ATIRADEISK | 57 | 137 | |
| | N-Term | acetylation | *ATIRADEISKIIR | 59 | | |
| | K25 | acetylation | EVKVVNTGTVLQVGDGIAR | 78 | 99 | |
| | K114 | acetylation | VINALAKPIDGRGEITASESR | 19 | 42 | |
| | K114 | acetylation | VINALAKPIDGR | 52 | | |
| | T181/183 | phosphorylation | TAVATDTILNQQGQNVICVYVAIGQK | 168 | 133 | |
| | K266 | acetylation | HTLIIYDDLSKQAQAYR | 42 | 95 | |
| | K374 | acetylation | VGSAAQIKAMK | 66 | 59 | |
| | K384 | acetylation | LKLELAQFAELEAFAQFASDLDKATQNQLAR | | 239 | |
| | K422 | acetylation | ELLKQPQSAPLTVEEQVMTIYTGTNGYLDSLELDQVR | 17 | 63 | |
| | K422 | acetylation | ELLKQPQSAPLTVEEQVMTIYTGTNGYLDSLELDQVRK | 40 | 68 | |
| | Y445 | phosphorylation | QPQSAPLTVEEQVMTIYTGTNGYLDSLELDQVR | | 40 | |
| | K456 | acetylation | KYLVELR | 43 | 101 | Schmidt et al. |
| | K466 | acetylation | TYVKTNKPEFQEIISSTK | 60 | 70 | |
| | K469 | acetylation | TYVKTNKPEFQEIISSTK | 49 | 61 | |
| | K469 | acetylation | TNKPEFQEIISSTK | 72 | 75 | |
| | K480 | acetylation | TNKPEFQEIISSTKTFTEEAEALLK | 31 | | |
| β | N-Term | acetylation | *MRINPTTSDPGVSTLEK | 74 | 125 | |
| | N-Term | acetylation | *MRINPTTSDPGVSTLEKK | 54 | 86 | |
| | K17 | acetylation | INPTTSDPGVSTLEKK | | 66 | |
| | K18 | acetylation | INPTTSDPGVSTLEKK | 100 | | |
| | K50 | acetylation | MPNIYNALIVK | 50 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| β | T54 | phosphorylation | DTAGQPMNVTCEVQQLLGNNR | 106 | 101 | Schmidt et al. |
| | T54 | phosphorylation | GRDTAGQPMNVTCEVQQLLGNNR | 69 | 144 | Schmidt et al. |
| | T54 | phosphorylation | DTAGQPMNVTCEVQQLLGNNRVR | | 93 | |
| | T62 | phosphorylation | DTAGQPMNVTCEVQQLLGNNR | 62 | 100 | |
| | T62 | phosphorylation | GRDTAGQPMNVTCEVQQLLGNNR | 64 | | |
| | K145 | acetylation | SAPAFTQLDTKLSIFETGIK | 38 | | |
| | K217 | acetylation | TREGNDLYMEMKESGVINEQNIAESK | | 90 | |
| | K359 | acetylation | GLAAKGIYPAVDPLDSTSTMLQPR | 52 | | |
| | K392 | acetylation | VKETLQR | 43 | 85 | Schmidt et al. |
| | K399 | acetylation | YKELQDIIAILGLDELSEEDRLTVAR | | 215 | |
| γ | K55 | acetylation | DRIGSVK | 25 | | |
| | K65 | acetylation | ITEAMKLVAAAK | | 49 | |
| | K71 | acetylation | LVAAAKVR | 36 | 59 | |
| | K117 | acetylation | KVALMVVTGDR | 66 | 47 | |
| | K150 | acetylation | KLGVDYTIISIGK | 87 | 122 | Schmidt et al. |
| | K150 | acetylation | KLGVDYTIISIGKK | 88 | 155 | Schmidt et al. |
| | K162/163 | acetylation | KLGVDYTIISIGKK | 54 | | |
| | K163 | acetylation | KGNTYFIR | 46 | 110 | |
| | S223 | phosphorylation | SDPVIHTLLPLSPK | 60 | 66 | |
| | S234 | phosphorylation | SDPVIHTLLPLSPK | 48 | 88 | |
| | K335 | acetylation | KTLSINYNR | 70 | 172 | |
| δ | S97 | phosphorylation | YASALADVADVTGTLEATNSDVEK | 153 | 228 | Schmidt et al. |
| | K101 | acetylation | YASALADVADVTGTLEATNSDVEKLIR | | 44 | |
| | T175 | phosphorylation | ITGTEVAVVTSVVKLENDHLAQIAK | 82 | | |
| | K196 | acetylation | LENDHLAQIAKGVQK | 37 | 96 | |
| | S216 | phosphorylation | IKTVIDPSLVAGFTIRYGNEGSK | 64 | | |
| ε | N-Term | acetylation | *TLNLCVLTPNR | 80 | 170 | |
| | N-Term | acetylation | *TLNLCVLTPNRSIWNSEVK | | 136 | |
| | T2 | phosphorylation | TLNLCVLTPNR | 68 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ε | S85 | phosphorylation | GSDIDPQEAQQTLEIAEANLR | 103 | 82 | |
| | K112 | acetylation | QKIEANLALR | 57 | 105 | Schmidt et al. |
| | K112 | acetylation | RQKIEANLALR | 20 | 106 | Schmidt et al. |
| | K112 | acetylation | QKIEANLALRR | 57 | 73 | Schmidt et al. |
| I | K73 | acetylation | GKAIEQLEK | 49 | 89 | |
| | K86/87 | acetylation | LKKVEMDADQFR | | 54 | |
| | K87 | acetylation | KVEMDADQFR | 82 | 103 | Schmidt et al. |
| | K87 | acetylation | KVEMDADQFRVNGYSEIER | 79 | 60 | Schmidt et al. |
| | K107 | acetylation | EKMNLINSTYK | 78 | 94 | |
| | K125 | acetylation | TLEQFENYKNETIQFEQQK | 41 | 116 | |
| | K135 | acetylation | TLEQFENYKNETIQFEQQKAINQVR | 96 | 94 | Schmidt et al. |
| | T155 | phosphorylation | VFQQALQGALGTLNSCLNNELHLR | 73 | 147 | |
| II | K124 | acetylation | DASIKEQLSGVK | 75 | 106 | |
| | K124 | acetylation | DASIKEQLSGVKDTSSEVK | 63 | 112 | |
| | K131 | acetylation | EQLSGVKDTSSEVK | 67 | 125 | Schmidt et al. |
| | K164 | acetylation | KETQLEVEAK | | 86 | |
| | K173 | acetylation | KETQLEVEAKLAEGR | | 63 | |
| | K138 | acetylation | DTSSEVKQLEEQANAVMR | 83 | | |
| | K161 | acetylation | AEISAALNKMK | | 52 | |
| | K163 | acetylation | AEISAALNKMK | 64 | | |
| | K173 | acetylation | ETQLEVEAKLAEGR | 58 | | |
| | K180 | acetylation | KKIEVELQEALGSLEQQKEDTIK | 73 | | |
| | K180 | acetylation | KIEVELQEALGSLEQQKEDTIK | 40 | 135 | |
| | S205 | phosphorylation | SLDSQISALSDDIVKK | | 44 | |
| | S205 | phosphorylation | SLDSQISALSDDIVKK | 70 | 98 | |
| | S208 | phosphorylation | SLDSQISALSDDIVKK | 62 | | |
| IV | K156 | acetylation | KGLGYFGK | 46 | 130 | |

**Table S2: Comparative cross-linking of naturally modified and deacetylated chloroplast ATP synthase.** The cross-linked protein subunits, the cross-linked residues, the number of obtained spectra and the ratio of naturally modified (d0) versus deacetylated (d4) chloroplast ATP synthase are given. Changes in protein interactions > 2-fold are highlighted in grey.

| Protein 1 | Protein 2 | Residue 1 | Residue 2 | # spectra | d0/d4 |
|---|---|---|---|---|---|
| α | α | 11 | 25 | 7 | 0.94 |
| | | 11 | 114 | 1 | 0.32 |
| | | 11 | 466 | 1 | 1.21 |
| | | 25 | 114 | 4 | 1.76 |
| | | 374 | 378 | 3 | 1.09 |
| | | 378 | 384 | 1 | 0.86 |
| | | 456 | 466 | 1 | 1.31 |
| | | 456 | 469 | 1 | 0.56 |
| | | 456 | 491 | 2 | 0.92 |
| | | 469 | 491 | 1 | 0.64 |
| α | β | 11 | 17 | 1 | 1.71 |
| | | 11 | 392 | 4 | 1.40 |
| | | 374 | 426 | 1 | 1.17 |
| | | 382 | 495 | 1 | 0.77 |
| | | 456 | 359 | 3 | 1.04 |
| | | 469 | 359 | 1 | 1.33 |
| α | δ | 2 | 101 | 8 | 0.99 |
| α | I | 2 | 125 | 6 | 0.67 |
| | | 114 | 135 | 15 | 0.68 |
| | | 466 | 116 | 1 | 0.71 |
| α | II | 456 | 164 | 1 | 0.59 |
| β | β | 18 | 50 | 11 | 1.10 |
| | | 359 | 392 | 3 | 0.74 |
| | | 359 | 399 | 4 | 0.52 |
| | | 359 | 426 | 2 | 0.73 |
| | | 392 | 399 | 3 | 0.44 |
| | | 392 | 426 | 5 | 1.52 |
| β | δ | 18 | 205 | 3 | 1.09 |
| | | 359 | 210 | 1 | 1.38 |
| β | γ | 359 | 55 | 1 | 1.05 |
| | | 392 | 55 | 2 | 2.00 |
| | | 426 | 71 | 2 | 1.20 |
| | | 426 | 149 | 1 | 0.91 |
| β | I | 426 | 80 | 1 | 1.02 |
| β | II | 426 | 131 | 1 | 2.56 |
| δ | δ | 210 | 231 | 1 | 1.33 |
| ε | ε | 2 | 20 | 2 | 7.17 |
| | | 105 | 112 | 2 | 204.21 |
| ε | III | 112 | 48 | 6 | 1.22 |

| | | | | | |
|---|---|---|---|---|---|
| γ | γ | 55 | 260 | 1 | 2.15 |
| | | 55 | 334 | 2 | 0.76 |
| | | 55 | 335 | 4 | 0.95 |
| | | 59 | 334 | 1 | 0.76 |
| | | 140 | 163 | 1 | 1.65 |
| | | 140 | 222 | 1 | 1.26 |
| | | 140 | 245 | 5 | 1.15 |
| | | 140 | 263 | 3 | 1.46 |
| | | 222 | 245 | 6 | 0.71 |
| | | 245 | 260 | 1 | 0.94 |
| I | I | 80 | 87 | 8 | 1.49 |
| I | II | 73 | 131 | 12 | 0.79 |
| | | 73 | 138 | 7 | 0.73 |
| | | 107 | 161 | 2 | 1.00 |
| | | 107 | 173 | 25 | 1.33 |

**Table S3: Comparative cross-linking of naturally modified and deacetylated/dephosphorylated chloroplast ATP synthase.** The cross-linked protein subunits, the cross-linked residues, the number of obtained spectra and the ratio of naturally modified (d0) versus deacetylated (d4) chloroplast ATP synthase are given.

| Protein 1 | Protein 2 | Residue 1 | Residue 2 | # spectra | d0/d4 |
|---|---|---|---|---|---|
| α | α | 378 | 491 | 1 | 2.08 |
| α | beta | 382 | 495 | 1 | 4.17 |
| α | delta | 11 | 101 | 1 | 2.77 |
| α | I | 2 | 125 | 1 | 2.96 |
| β | β | 18 | 50 | 4 | 5.45 |
| | | 359 | 17 | 1 | 0.25 |
| | | 359 | 392 | 4 | 5.76 |
| δ | δ | 101 | 200 | 1 | 3.86 |
| | | 200 | 210 | 1 | 1.88 |
| γ | γ | 55 | 335 | 3 | 1.20 |
| | | 140 | 222 | 2 | 3.17 |
| | | 140 | 245 | 1 | 8.23 |
| | | 222 | 245 | 2 | 1.77 |
| I | I | 80 | 87 | 12 | 3.55 |
| | | 87 | 107 | 1 | 1.00 |
| I | II | 73 | 131 | 12 | 5.01 |
| | | 73 | 138 | 7 | 3.28 |
| | | 107 | 161 | 1 | 4.27 |
| | | 107 | 173 | 6 | 1.71 |

**Table S4: Abundance of identified PTMs in the cATPase.** The Stoichiometries of the modified sites were obtained using MaxQuant software. The protein subunit, the modified residue/site, the peptide sequence, the ratio of unmodified vs modified peptides (intensities) and the percentage of the modification as well as the average in three replicates are given. Note that some modified sites identified in this study (**Table S1**) could not be quantified.

| Subunit | Site | Peptide sequence | Ratio unmodified/modified | | | Per cent modified | | | Average |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 1 | 2 | 3 | |
| α | *ac*K469 | TN**K**PEFQEIISSTK | 40.60 | 13.23 | 6.45 | 2.40 | 7.03 | 13.42 | 7.62 |
| | *ac*K456 | **K**YLVELR | | 19.05 | 1348.67 | | 4.99 | 0.07 | 1.69 |
| | *ac*K466 | TYV**K**TNKPEFQEIISSTK | 119.14 | 26.34 | | 0.83 | 3.66 | | 1.50 |
| | *ac*K422 | ELL**K**QPQSAPLTVEEQVMTIYTGTNGYLDSLELDQVRK | 56.55 | | 89.90 | 1.74 | | 1.10 | 0.95 |
| | *ac*K384 | L**K**LELAQFAELEAFAQFASDLDKATQNQLAR | 42.71 | | | 2.29 | | | 0.76 |
| | *ac*K266 | HTLIIYDDLS**K**QAQAYR | 116.73 | | | 0.85 | | | 0.28 |
| | *ac*K25 | EV**K**VVNTGTVLQVGDGIAR | 301.50 | | | 0.33 | | | 0.11 |
| β | *ac*K392 | V**K**ETLQR | 0.05 | | | 95.55 | | | 31.85 |
| | *ac*K217 | TREGNDLYMEM**K**ESGVINEQNIAESK | 180.33 | | | 0.55 | | | 0.18 |
| | *ac*K17 | INPTTSDPGVSTLE**K**K | | 335.50 | | | 0.30 | | 0.10 |
| | *ac*K399 | Y**K**ELQDIIAILGLDELSEEDRLTVAR | | | 1236.28 | | | 0.08 | 0.03 |
| γ | *ac*K335 | **K**TLSINYNR | | 189.59 | 55.29 | | 0.52 | 1.78 | 0.77 |
| | *ac*K163 | **K**GNTYFIR | | 93.91 | 125.73 | | 1.05 | 0.79 | 0.61 |
| | *ac*K150 | **K**LGVDYTIISIGKK | 616.52 | 339.97 | 270.73 | 0.16 | 0.29 | 0.37 | 0.27 |
| | *ac*K71 | LVAAA**K**VR | 166.34 | | | 0.60 | | | 0.20 |
| | *ac*K65 | ITEAM**K**LVAAAK | | 220.41 | | | 0.45 | | 0.15 |
| | *ac*K117 | **K**VALMVVTGDR | | 711.29 | 521.51 | | 0.14 | 0.19 | 0.11 |
| δ | *ac*K196 | LENDHLAQIA**K**GVQK | | | 8.06 | | | 11.04 | 3.68 |
| | *ac*K101 | YASALADVADVTGTLEATNSDVE**K**LIR | | | 120.47 | | | 0.82 | 0.27 |
| ε | *ac*K112 | Q**K**IEANLALR | 1540.38 | 31.51 | 244.23 | 0.06 | 3.08 | 0.41 | 1.18 |
| I | *ac*K87 | **K**VEMDADQFR | | 391.42 | 60.27 | | 0.25 | 1.63 | 0.63 |
| | *ac*K107 | E**K**MNLINSTYK | | 704.23 | 73.15 | | 0.14 | 1.35 | 0.50 |
| | *ac*K86 / *ac*K87 | L**KK**VEMDADQFR | | | 434.31 | | | 0.23 | 0.08 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | *ac*K135 | TLEQFENYKNETIQFEQQ**K**AINQVR | | | 3460.93 | | | 0.03 | 0.01 |
| II | *ac*K164 | **K**ETQLEVEAK | 41.95 | 689.04 | 7.06 | 2.33 | 0.14 | 12.40 | 4.13 |
| | *ac*K161 | AEISAALN**K**MK | | | 30.62 | | | 3.16 | 1.05 |
| | *ac*K135 | TLEQFENY**K**NETIQFEQQK | | | 203.88 | | | 0.49 | 0.99 |
| | *ac*K173 | ETQLEVEA**K**LAEGR | | 37.14 | | | 2.62 | | 0.87 |
| | *ac*K180 | **K**IEVELQEALGSLEQQKEDTIK | | 730.67 | 120.73 | | 0.14 | 0.82 | 0.32 |
| | *ac*K124 | DASI**K**EQLSGVK | | 543.69 | 618.62 | | 0.18 | 0.16 | 0.11 |
| | *ac*K131 | EQLSGV**K**DTSSEVK | | 4584.63 | 4414.43 | | 0.02 | 0.02 | 0.01 |
| IV | *ac*K156 | **K**GLGYFGK | | 1630.18 | 595.45 | | 0.06 | 0.17 | 0.08 |
| α | *p*T181 | TAVA**T**DTILNQQGQNVICVYVAIGQK | 0.73 | 0.66 | | 1.37 | 1.54 | | 0.51 |
| | *p*T183 | TAVATD**T**ILNQQGQNVICVYVAIGQK | | | | | | | 0.46 |
| β | *p*T62 | GRDTAGQPMNV**T**CEVQQLLGNNR | | 134.58 | 25.20 | | 0.007 | 0.040 | 0.02 |
| | *p*T54 | D**T**AGQPMNVTCEVQQLLGNNR | | | 1245.35 | | | 0.0008 | 0.0003 |
| γ | *p*S234 | SDPVIHTLLPL**S**PK | | 9324.88 | 6716.37 | | 0.0001 | 0.0001 | 0.0001 |
| δ | *p*S97 | YASALADVADVTGTLEATN**S**DVEK | | 592.45 | | | 0.002 | | 0.001 |
| I | *p*T155 | VFQQALQGALG**T**LNSCLNNELHLR | 0.40 | 698.03 | 8.10 | 2.51 | 0.001 | 0.12 | 0.88 |
| II | *p*S205 | SLD**S**QISALSDDIVKK | 4398.89 | 15209.36 | | 0.0002 | 0.00007 | | 0.0001 |
| | *p*S208 | SLDSQI**S**ALSDDIVKK | | 43630.02 | | | 0.00002 | | 0.00001 |

**Figures**

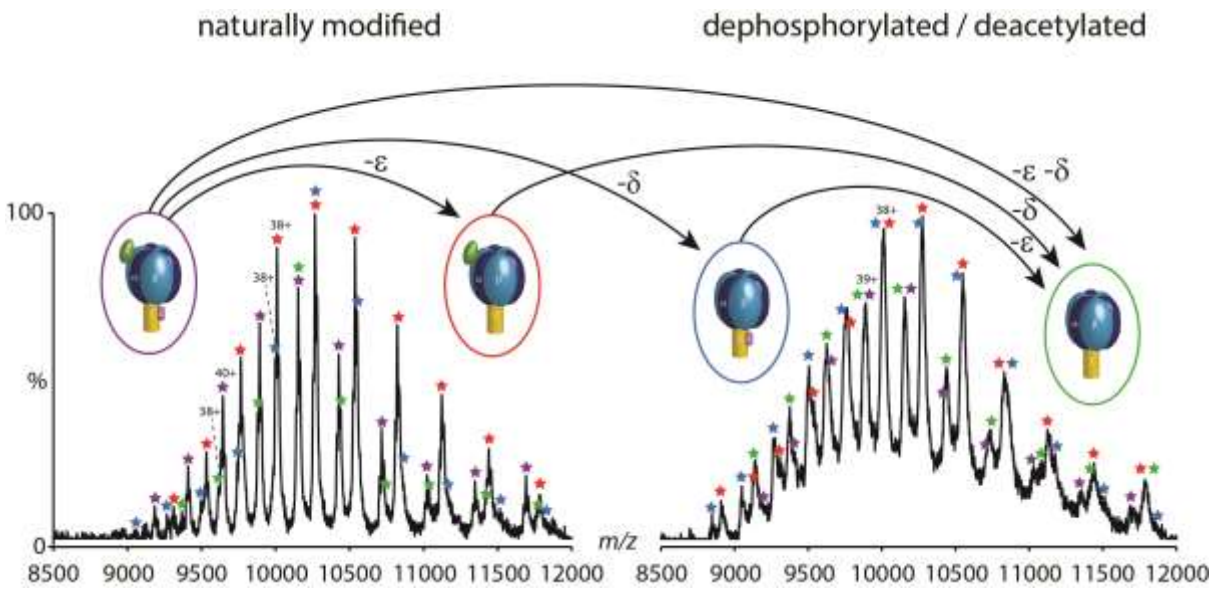


**Figure S1: Simultaneous dehosphorylation and deacetylation.** Spectra of the naturally modified **(lhs)** and the dephosphorylated/deacetylated **(rhs)** cATPase are shown. Four subcomplexes of cATPase were identified.

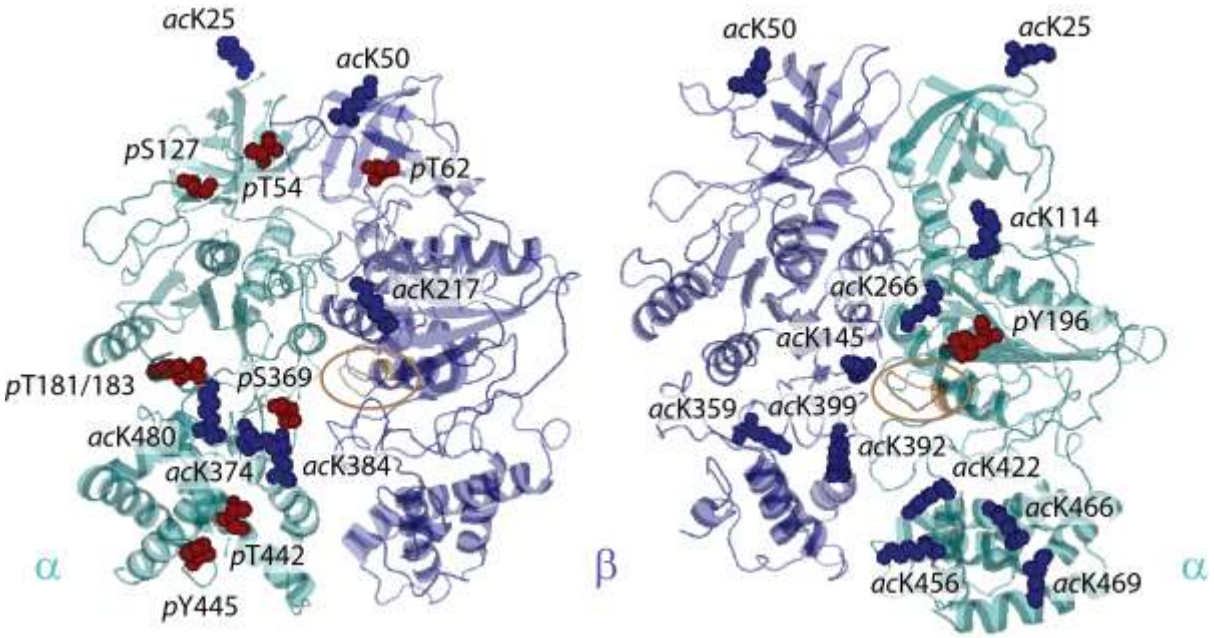


**Figure S2: Phosphorylation and acetylation sites are located at the protein interfaces.** The α/β and β/α interfaces of the cATPase are shown (PDB ID XY). Phosphorylated (red) and acetylated (blue) residues are shown (space fillings). Most phosphorylation sites are located in the α/β interface, while acetylation is more prevalent in the β/α interface.