Supplementary material for:

Competition between electrostatic interactions and halogen bonding in the protein-ligand system: structural and thermodynamic studies of 5,6-dibromobenzotriazole - hCK2α complexes.

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Supplementary Materials

Buffers:

Buffers used for crystallization:

Protein buffer:
25 mM Tris-HCl pH 8.5, 0.5 M NaCl, 5 mM β-mercaptoethanol

Reservoir buffers:
20 mM sodium formate, 20 mM ammonium acetate, 20 mM sodium citrate tribasic dihydrate, 20 mM sodium potassium tartrate tetrahydrate, 20 mM sodium oxamate, 20% polyethylene glycol 550 monomethyl ester, 10% polyethylene glycol 20 000, and 0.1 M buffering solution of either:
  imidazole/MES pH 5.5
  imidazole/MES pH 6.5
  sodium HEPES/MOPS pH 7.5
  Tris/Bicine pH 8.5
  CAPSO/NaOH pH 9.5

Buffers used in ITC experiments:

25 mM Bis-Tris Propane 0.5 M NaCl and 1% DMSO; pH 6.5-8.7
25 mM MOPS, 0.5 M NaCl and 1% DMSO; pH 6.5 and 6.7
25 mM MES, 0.5 M NaCl and 1% DMSO; pH 6.5 and 6.7
25 mM BES, 0.5 M NaCl and 1% DMSO; pH 6.5, 6.7 and 7.0
25 mM PIPES, 0.5 M NaCl and 1% DMSO; pH 6.5, 6.7, 7.0 and 7.5
25 mM Tris-HCl, 0.5 M NaCl and 1% DMSO; pH 7.0, 7.5, 8.0, 8.5 and 8.7
25 mM HEPES, 0.5 M NaCl and 1% DMSO; pH 7.0, 7.5 and 8.0
25 mM Tricine, 0.5 M NaCl and 1% DMSO; pH 8.0, 8.5 and 8.7
25 mM TES, 0.5 M NaCl and 1% DMSO; pH 8.0
25 mM CHES, 0.5 M NaCl and 1% DMSO; pH 8.7
25 mM AMPSO, 0.5 M NaCl and 1% DMSO; pH 8.5 and 8.7

Buffers used in HDX experiments:

H2O Reaction buffer: 25 mM Tris-HCl, 0.5 M NaCl
H2O Stop Buffer: 2 M glycine buffer, pH 2.5
D2O Reaction buffer: 25 mM Tris-DCl, 0.5 M NaCl pH 6.7 and pH 8.7
D2O Stop Buffer: 2 M glycine buffer, pH 2.5
Supplementary Tables

Table S1. Thermodynamic parameters of the 5,6-DBBt affinity to hCK2α determined with the aid of isothermal titration calorimetry (ITC) at different conditions. The data were collected in two or more independent experiments that were separately evaluated.

| pH  | Buffer | $\Delta G$ [kJ·mol$^{-1}$] | $K_d$ [nM] | $\Delta H$ [kJ·mol$^{-1}$] | $\Delta S$ [J·mol$^{-1}$·K$^{-1}$] |
|-----|--------|-----------------------------|-------------|-----------------------------|---------------------------------|
| 6.5 |        | -39.1±0.4                  | 143±24      | -57.6±1.4                   | -62±6                           |
|     |        | -38.8±0.9                  | 158±57      | -57.4±4.1                   | -62±16                           |
|     |        | -38.3±0.4                  | 197±30      | -55.6±1.4                   | -58±6                           |
| 6.7 |        | -40.1±0.5                  | 93±20       | -65.0±1.9                   | -83±8                           |
|     |        | -39.3±0.7                  | 128±36      | -59.2±2.4                   | -67±10                           |
|     |        | -39.7±0.7                  | 111±31      | -51.1±1.9                   | -38±8                           |
| 7.0 |        | -40.6±0.3                  | 76±9        | -56.2±0.8                   | -52±4                           |
|     |        | -40.8±0.3                  | 72±9        | -59.1±0.9                   | -61±4                           |
| 7.2 |        | -40.5±0.7                  | 80±24       | -60.4±2.4                   | -67±10                           |
|     |        | -40.2±0.6                  | 89±22       | -58.2±1.9                   | -60±8                           |
| 7.5 | Bis-Tris | -40.4±0.5               | 83±15       | -54.2±1.3                  | -46±6                           |
|     |        | -41.4±0.3                  | 57±6        | -57.0±0.7                   | -52±3                           |
| 7.7 |        | -41.8±1.3                  | 48±25       | -51.7±4.0                   | -33±17                           |
|     |        | -41.5±0.6                  | 54±14       | -53.8±1.4                   | -41±7                           |
| 8.0 |        | -41.6±0.4                  | 51±8        | -53.8±1.1                   | -41±5                           |
|     |        | -41.6±0.6                  | 51±11       | -53.8±1.9                   | -41±8                           |
| 8.2 |        | -41.9±0.3                  | 46±5        | -55.7±0.6                   | -46±3                           |
|     |        | -42.1±0.2                  | 43±3        | -54.4±0.4                   | -41±2                           |
| 8.5 |        | -42.5±0.5                  | 36±7        | -53.1±1.2                   | -36±5                           |
|     |        | -41.9±0.5                  | 46±8        | -52.2±1.1                   | -35±5                           |
| 8.7 |        | -41.6±0.3                  | 51±5        | -55.2±0.5                   | -46±3                           |
|     |        | -41.5±0.3                  | 54±6        | -53.2±0.6                   | -39±3                           |
| 6.5 | MES    | -39.7±0.5                  | 110±24      | -44.3±1.6                   | -15±7                           |
|     |        | -39.7±0.7                  | 112±30      | -42.8±2.0                   | -11±9                           |
| 6.5 | MOPS   | -39.3±0.4                  | 130±22      | -46.8±1.3                   | -25±6                           |
|     |        | -38.5±0.4                  | 182±32      | -47.0±1.4                   | -28±6                           |
| 6.5 | BES    | -40.7±0.8                  | 74±25       | -51.0±2.7                   | -35±12                          |
|     |        | -39.8±0.6                  | 107±25      | -49.2±1.8                   | -31±8                           |
| 6.5 | PIPES  | -38.9±0.9                  | 151±56      | -46.3±3.5                   | -7±4                            |
|     |        | -39.4±0.8                  | 126±39      | -39.5±2.0                   | 0±9                             |
| 6.7 | MES    | -38.6±0.3                  | 173±24      | -48.8±1.2                   | -34±5                           |
|     |        | -39.5±1.7                  | 118±8       | -44.6±6.6                   | -17±27                          |
| 6.7 | MOPS   | -40.3±0.5                  | 88±19       | -49.5±1.3                   | -31±6                           |
|     |        | -40.0±0.9                  | 98±36       | -52.3±3.0                   | -34±12                          |
| 6.7 | BES    | -39.5±0.3                  | 121±14      | -53.1±0.9                   | -46±4                           |
|     |        | -40.3±0.7                  | 87±24       | -52.2±2.1                   | -40±9                           |
| 6.7 | PIPES  | -40.2±1.1                  | 89±38       | -34.5±2.6                   | -11±12                          |
|     |        | -39.0±0.9                  | 148±53      | -46.1±2.8                   | -24±12                          |
| 7.0 | Tris   | -41.5±0.5                  | 54±11       | -57.2±1.7                   | -53±7                           |
|     |        | -40.9±0.4                  | 67±12       | -58.4±1.3                   | -59±6                           |
| 7.0 | PIPES  | -40.0±0.4                  | 98±16       | -43.9±1.0                   | -13±4                           |
|     |        | -41.9±0.7                  | 45±12       | -43.7±1.2                   | -6±6                            |
| pH  | Buffer   | ionic strength (mM) | voltage (mV) | capacity (nF) |
|-----|----------|---------------------|--------------|--------------|
| 7.0 | HEPES    | 40.7±0.4            | -46.8±0.9    | -20±4        |
|     |          | 41.5±0.5            | -48.0±1.1    | -22±5        |
| 7.0 | BES      | 40.6±0.5            | -46.7±1.2    | -20±5        |
|     |          | 40.3±0.8            | -50.0±2.5    | -33±11       |
| 7.5 | Tris     | 41.7±0.3            | -58.4±0.8    | -56±4        |
|     |          | 42.0±0.6            | -58.6±1.6    | -55±7        |
| 7.5 | PIPES    | 41.7±0.4            | -52.4±0.9    | -36±4        |
|     |          | 41.6±0.5            | -50.4±1.2    | -30±5        |
| 7.5 | HEPES    | 42.2±0.4            | -52.0±0.8    | -36±4        |
|     |          | 40.5±0.6            | -53.5±2.1    | -44±9        |
| 7.5 | BES      | 40.9±0.3            | -53.4±0.7    | -42±3        |
|     |          | 41.9±0.4            | -55.0±0.9    | -44±4        |
| 8.0 | Tris     | 41.7±0.4            | -57.4±1.1    | -53±5        |
|     |          | 43.2±0.4            | -58.5±1.2    | -51±5        |
| 8.0 | Tricine  | 42.0±0.4            | -52.1±0.8    | -34±4        |
|     |          | 42.1±0.4            | -55.2±1.0    | -44±5        |
| 8.0 | HEPES    | 41.1±0.6            | -54.5±1.9    | -45±8        |
|     |          | 41.8±0.5            | -55.6±1.4    | -46±6        |
| 8.0 | TES      | 41.3±0.3            | -55.7±0.6    | -48±3        |
|     |          | 41.3±0.4            | -57.3±1.3    | -53±6        |
| 8.5 | Tris     | 41.8±0.6            | -51.8±1.7    | -34±8        |
|     |          | 42.4±0.6            | -52.6±1.4    | -34±6        |
| 8.5 | Tricine  | 42.8±0.7            | -51.3±1.4    | -29±7        |
|     |          | 42.7±0.5            | -51.3±1.0    | -29±5        |
| 8.5 | AMPSO    | 43.8±0.6            | -51.0±1.0    | -24±5        |
|     |          | 41.9±0.5            | -51.9±1.1    | -33±5        |
| 8.7 | Tris     | 41.3±0.5            | -53.1±1.1    | -40±5        |
|     |          | 41.2±0.4            | -53.9±1.0    | -43±5        |
| 8.7 | Tricine  | 41.2±0.4            | -53.9±1.0    | -47±5        |
|     |          | 41.5±0.5            | -54.0±1.4    | -42±6        |
| 8.7 | AMPSO    | 42.1±0.5            | -51.6±1.1    | -32±5        |
|     |          | 42.8±0.8            | -52.6±2.1    | -33±9        |
| 8.7 | CHES     | 43.3±1.0            | -55.0±2.6    | -39±11       |
|     |          | 42.0±0.5            | -51.9±1.2    | -33±5        |
|     |          | 43±9                | -51.9±1.2    | -33±5        |
| 1-CH$_3$-5,6-DBBt | 33.2±1.0      | 1520±600          | -7.0±0.8     | 88±5         |
|     |          | 32.9±1.7            | 1710±120      | -7.8±2.3     | 84±13        |
**Table S2. Data collection statistics for all crystals of the presented complexes.** Published data \(^2\) are shaded in red, data deposited in the PDB for the current paper – in gray, the unpublished repetitions are in white.

|                      | hCK2 - 5,6-DBBt | hCK2 - 5,6-DBBt | hCK2 - 5,6-DBBt | hCK2 - 5,6-DBBt | hCK2 - 4,5,6,7-TBBt | hCK2 - 4,5,6,7-TBBt | hCK2 - 4,5,6,7-TBBt | hCK2 - 4,5,6,7-TBBt | hCK2 - 4,5,6,7-TBBt |
|----------------------|-----------------|-----------------|-----------------|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| **pH**               | 5.5             | 6.5             | 7.5             | 7.5             | 8.5                 | 7.5                 | 7.5                 | 7.5                 | 8.5                 |
| **Inhibitor**        | cocryrst.       | cocryrst.       | cocryrst.       | cocryst. soaked | cocryst. soaked     | cocryst. soaked     | cocryst. soaked     | cocryst. soaked     | cocryst. soaked     |
| **Mg\(^{2+}\)**     | 4 mM            | 4 mM            | 4 mM            | -               | 4 mM                | -                   | 4 mM                | 4 mM                | 4 mM                |
| **2nd binding site** | no              | yes (weak)      | no              | yes (2 conf.)   | yes                 | yes                 | yes                 | yes (weak)          | yes (very weak)     | yes (very weak)     |
| **Space group**      | \(P_{4_2}2_1/2\) | \(P_{4_2}2_1/2\) | \(P_{4_2}2_1/2\) | \(P_{4_2}2_1/2\) | \(P_{4_2}2_1/2\)    | \(P_{4_2}2_1/2\)    | \(P_{4_2}2_1/2\)    | \(P_{4_2}2_1/2\)    | \(P_{4_2}2_1/2\)    |
| **Cell dimensions**  |                 |                 |                 |                 |                     |                     |                     |                     |                     |
| \(a, b (\text{Å})\) | 127.5           | 128.5           | 127.2           | 128.4           | 128.1               | 129.5               | 127.4               | 128.5               | 128.5               |
| \(c (\text{Å})\)    | 61.0            | 61.2            | 60.9            | 124.2           | 61.0                | 60.9                | 61.0                | 61.3                | 61.3                |
| **Wavelength (Å)**  | 0.9117          | 0.9117          | 0.9117          | 0.9150          | 0.9150              | 0.9116              | 0.9116              | 0.9117              | 0.9117              |
| **Beamline**         | DESY            | DESY            | DESY            | BESY            | P11                 | DESY                | P11                 | DESY                | P11                 |
| **Resolution (Å)**   | 2.55 (45.1 – 7.755) | 2.58 (45.4 – 7.64) | 1.93 (36.2 – 5.72) | 2.14 (45.4 – 6.36) | 2.25 (45.3 – 6.68) | 2.30 (45.8 – 6.84) | 1.88 (45.0 – 5.60) | 1.95 (40.0 – 5.83) | 1.95 (20.0 – 2.19) |
| **lowest shell**     |                 |                 |                 |                 |                     |                     |                     |                     |                     |
| **highest shell**    |                 |                 |                 |                 |                     |                     |                     |                     |                     |
| **R\(_{\text{_meas}}\) (%)\(^3\)** | 30.4 (9.4, 135.9) | 31.4 (6.2, 210.5) | 14.2 (3.8, 170.3) | 13.6 (3.8, 176.4) | 24.7 (5.3, 187.2) | 11.2 (3.9, 87.3) | 9.1 (5.5, 127.8) | 11.0 (5.7, 150.2) | 24.1 (5.2, 187.6) |
| **CC\(_{1/2}\) (%)** | 99.5 (99.9, 80.3) | 99.6 (99.9, 68.6) | 99.9 (100, 80.7) | 100 (100, 71.0)  | 99.8 (100, 72.4)  | 99.9 (99.9, 85.1) | 99.9 (99.9, 83.9) | 99.9 (100, 72.3)  | 100 (100, 84.5)  |
| **I/σI\(^4\)**      | 10.7 (31.7, 2.03) | 12.6 (43.4, 1.99) | 22.7 (82.5, 1.98) | 22.2 (69.1, 2.03) | 15.4 (56.5, 1.98) | 27.7 (80.2, 4.24) | 20.6 (51.1, 2.07) | 18.5 (49.2, 2.07) | 15.2 (46.1, 2.03) |
| **Completeness (%)** | 99.9 (99.3, 99.8) | 99.8 (99.3, 99.8) | 99.9 (99.4, 99.7) | 99.9 (99.3, 99.1) | 99.9 (99.5, 99.2) | 99.2 (99.7, 99.4) | 99.6 (98.8, 99.5) | 99.9 (99.1, 99.8) | 99.9 (99.5, 99.5) |
| **Multiplicity\(^5\)** | 26.3 (23.0, 26.9) | 26.0 (25.3, 26.4) | 26.2 (23.3, 23.4) | 26.7 (23.0, 23.7) | 26.3 (23.1, 25.3) | 25.4 (22.3, 26.1) | 24.8 (21.3, 23.6) | 24.6 (21.7, 23.5) | 25.9 (22.0, 25.2) |
| **Number of reflections** | 16932           | 16669           | 38226           | 57785           | 24732              | 23379              | 41198              | 37408              | 14815              |

\(^1\) Lowest and highest shell in brackets. \(^2\) Data cut due to an ice ring.
Table S3. Characterization of short halogen...oxygen contacts identified in the newly determined structures.

| PDB  | pH | XB donor     | XB acceptor  | Br…Acc distance (Å) | C:Br…Acc angle (deg) |
|------|----|--------------|--------------|---------------------|----------------------|
| 7QGC | 8.5| Br5 (DBBt)   | O (E114)     | 3.95                | 161                  |
| 7QGC | 8.5| Br6 (DBBt)   | OD1 (N 118)  | 4.44                | 139                  |
| 7QGB | 6.5| Br5 (DBBt)   | O (E114)     | 4.03                | 161                  |
| 7QGB | 6.5| Br6 (DBBt)   | OD1 (N 118)  | 4.57                | 142                  |
| 7QGD | 5.5| Br5 (DBBt)   | O (E114)     | 4.05                | 159                  |
| 7QGD | 5.5| Br6 (DBBt)   | OD1 (N 118)  | 4.47                | 147                  |
| 7QGE | 8.5| Br4 (TBBt)   | O(V116)      | 3.08                | 169                  |
| 7QGE | 8.5| Br5 (TBBt)   | O(E114)      | 3.94                | 163                  |
| 7QGE | 8.5| Br6 (TBBt)   | Ring center (F113) | 3.84 | 160 |
Table S4. Poses of benzotriazole related compounds in the kinase pockets. The compounds with large modification at position 1 and modified at position 2 have been eliminated, due to the steric hindrance preventing the potential contact with the lysine (K). K - the lysine at the bottom of the pocket corresponding to K68 in hCK2α. pKa\textsuperscript{calc} - predicted pKa of deprotonation with MolGpKa service \textsuperscript{1}.

| PDB | pKa\textsuperscript{exp}/pKa\textsuperscript{calc} | Predicted 5-mem. ring status at pH 7.5 in solution | Proximity | 5-membered ring constitution | Compound | Kinase | Cryst. buffer pH |
|-----|----------------------|-----------------------------------------------|----------|----------------------------|----------|-------|-----------------|
| 1PSE | 4.78/4.1 charged | hinge | NNN | 4,5,6,7-tetramethoxymethoxybenzimidazole | CDK2 | 7 |
| 1J91 | 4.78/4.1 charged | K | NNN | 4,5,6,7-tetramethoxymethoxybenzimidazole | mCK2α | 8.5 |
| 6TLL | 4.78/4.1 charged | hinge/K | NNN | 4,5,6,7-tetramethoxymethoxybenzimidazole | hCK2α | 7.5 |
| 6TLR | 5.84/6.4 charged | hinge | NNN | 4,6-dibromobenzimidazole | hCK2α | 7.5 |
| 6TLO | 5.91/5.1 charged | K/hinge | NNN | 4,5,6-trimethoxymethoxybenzimidazole | hCK2α | 7.5 |
| 6TLS | 6.38/6.3 charged | hinge | NNN | 4,6-dibromobenzimidazole | hCK2α | 7.5 |
| 6TLU | 6.49/6.1 charged | K | NNN | 4,5-dibromobenzimidazole | hCK2α | 7.5 |
| 6TLP | 6.93/6.9 charged | K | NNN | 5,6-dibromobenzimidazole | hCK2α | 7.5 |
| 5TS8 | 6.93/6.9 charged | K/hinge | NNN | 5,6-dibromobenzimidazole | mCK2α | 7.5 |
| 6TLV | 7.55/7.5 charged/ neutral | K | NNN | 5-bromobenzimidazole | hCK2α | 7.5 |
| 6TLW | 7.08/7.2 charged | K | NNN | 4-bromobenzimidazole | hCK2α | 7.5 |
| 7AIZ | /4.6 charged | K | NNN | 6-bromo-5-chloro-triazolo[4,5-b]pyridine | hCK2α | 8.5 |
| 7AIB | /4.8 charged | K | NNN | 5,6-dibromo-triazolo[4,5-b]pyridine | hCK2α | 8.5 |
| 7A22 | /3.6 charged | hinge | NNN | 5,6,7-trimethoxymethoxybenzimidazole | hCK2α | 8.5 |
| 7A49 | /4.6 charged | K | NNN | 6-bromo-5-chloro-triazolo[4,5-b]pyridine | hCK2α | 5.5 |
| 7A4B | /4.8 charged | K | NNN | 5,6,7-trimethoxymethoxybenzimidazole | hCK2α | 5.5 |
| 7A4C | /3.6 charged | hinge | NNN | 5,6,7-trimethoxymethoxybenzimidazole | hCK2α | 5.5 |
| 3KXG | /6.2 charged | hinge | NNC | 3,4,5,6,7-pentamethylamidazole | mCK2α | 8 |
| 3KXN | /7.0 charged | hinge | NCN | 4,5,6,7-tetramethylamidazole | mCK2α | 8 |
| 7A2H | /7.3 charged | hinge | NCN | 5,6,7-trimethylamidazole | hCK2α | 8.5 |
| 20XY | /10.9 neutral | hinge | NCN | 4,5,6,7-tetramethylamidazole | mCK2α | 8 |
| 3PVG | /3.2\textsuperscript{a} neutral | hinge | NCN | (4,5,6,7-tetramethylamidazole-1-yl)acetic acid | mCK2α | 8 |
| 3H30 | /13 neutral | hinge | NCN +N1-modified | 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole | hCK2α | 5.6 |
| 3OFM | /13.6 neutral | hinge | NNN +N1-modified | 3-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)propan-1-ol | hCK2α | 8.5 |
| 3RPS | /13.6 neutral | hinge | NNN +N1-modified | 3-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)propan-1-ol | hCK2α | 8.5 |
| 3HMQ | /13.6 neutral | hinge | NNN +N1-modified | 3-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)propan-1-ol | hCK2α | 8.5 |
| 7AT9 | /13.6 neutral | hinge | NNN +N1-modified | 3-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)propan-1-ol | hCK2α | 8.5 |
| 5OWH | neutral | hinge/K | NNC | (4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)propan-1-amine | hCK2α | 5.5 |
| 5OWL | neutral | hinge | NCN +N1-modified | (4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)propan-1-amine | hCK2α | 5.5 |
| 3MY1 | /13.0:12.9 neutral | hinge | NCN +N1-modified | 8-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)octanoic acid | CDK9 | 6.5 |
| 3MY5 | /13.0:12.9 neutral | hinge | NCN +N1-modified | 8-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)octanoic acid | CDK2 | 7 |
| 7ATV | /13.6:13.7 neutral | hinge | NCN +N1-modified | N-[2-[3,4-dichlorophenyl]ethyl]-N-[4-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)butyl]butanediame | hCK2α | 8.5 |
| 7AT5 | /13.6:13.7 neutral | hinge | NCN +N1-modified | N-[2-[3,4-dichlorophenyl]ethyl]-N-[4-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)butyl]butanediame | hCK2α | 8.5 |
| 6SPX | /4.7\textsuperscript{b} neutral | hinge | NCN +N1-modified | 8-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)octanoic acid | hCK2α | 5.6 |
| 6SPW | /4.5\textsuperscript{b} neutral | hinge | NCN +N1-modified | 8-(4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)octanoic acid | hCK2α | 5.6 |
| 4FBX | /-3\textsuperscript{c} neutral | hinge | NCN +N1-modified | ARC-1154 (bisubstrate conjugate of (4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)acetic acid | hCK2α | 5.25 |
| 4KWP | /13.0:13.3 neutral | hinge | NCN +N1-modified | 4,5,6,7-tetramethoxymethoxybenzimidazole-1-yl)acetic acid | hCK2α | 8.5 |

\textsuperscript{a} The benzimidazole ring is uncharged but the attached group is charged and forms an ionic pair with K68.

\textsuperscript{b} The attached group is a long chain charged at the end.

\textsuperscript{c} The attached peptide part is highly charged and reach the protein substrate binding site.
Suppl. Fig. 1. The interaction of TBBt with the ligand binding pocket of hCK2α. The dashed line indicates the proximity of H160 (modeled in two conformations) to the triazole ring of the ligand in one of its four poses at pH 7.5.
Suppl. Fig. 2. Model of 1CH₃-5,6-DBBt (A) and 2CH₃-5,6-DBBt (B) binding in hCK2α ATP-binding site. Modeling was performed with Yasara Structure package, using yasara2 force-field. Structure of 5,6-DBBt-hCK2α complex (7QGD) was used as the reference and original ligand was substituted with methylated analog. The procedure was restricted to the cuboid region that contained all residues from the ATP binding site. During simulations, the sidechain atoms of protein residues proximal to the location of the original ligand (8 Å threshold) were flexible, while coordinates of all other protein atoms were kept fixed.
Suppl. Fig. 3. The primary ligand binding site in complexes of hCK2α with 5,6-DBBt (top) and TBBt (bottom) at different pH values. The anomalous difference Fourier maps were calculated for the models without the ligands, contoured at 4.5 rmsd and shown in yellow. The structures obtained at pH 7.5 have been published before (PDB 6TLP and 6TLL) \(^2\). The replicas indicate that the occupation of the primary binding site is consistent in the crystals. Only the first presented structures at each pH value (mark with ticks) were deposited in the PDB, the replicas were not refined further. The location of the second hinge conformation in complexes with TBBt was ambiguous and traced only tentatively for chain completeness.
Suppl. Fig. 4. The binding mode of 5,6-DBBt in complex with hCK2α in different pH conditions. The anomalous difference Fourier maps were calculated for the models without the ligands, contoured at 4.5 rmsd and shown in yellow. The composite omit electron density maps are contoured at 1.5 rmsd and shown in gray, the difference density map is contoured at 3 rmsd (green) and -3 rmsd (red). The structure obtained at pH 7.5 has been published before (PDB 6TLP)\textsuperscript{2}. 

\textsuperscript{2} PDB 6TLP is a Protein Data Bank entry that provides structural information about the complex of 5,6-DBBt with hCK2α at pH 7.5. This reference is important for understanding the differences in binding modes across different pH conditions.
Suppl. Fig. 5. The binding mode of 4,5,6,7-TBBt in complex with hCK2α at two different pH values. The anomalous difference Fourier maps were calculated for the models without the ligands, contoured at 4.5 rmsd and shown in yellow (top panel). The composite omit electron density maps are contoured at 1 rmsd and shown in gray, the difference density map is contoured at 3 rmsd (green) and -3 rmsd (red) (bottom panel). The structure obtained at pH 7.5 has been published before (PDB 6TLL)².
Suppl. Fig. 6. The secondary ligand binding site in complexes of hCK2α with 5,6-DBBt (top) and TBBt (bottom) at different pH values. The anomalous difference Fourier maps were calculated for the models without the ligands, contoured at 2.5 rmsd and shown in yellow. The structures obtained at pH 7.5 have been published before (PDB 6TLP and 6TLL)\(^2\). The replicas indicate that the occupation of the secondary binding site is inconsistent in the crystals. Only the first presented structures at each pH value (marked with ticks) were deposited in the PDB, the replicas were not refined further. The position of the second ligand molecule in the crystals clashed with the protein in the region of residue 36 and thus the ligands were in most cases modelled at half occupancy only (to match one of the alternative conformations of the protein).
Fig. S7

Suppl. Fig. 7. Results of 10 sec hydrogen deuterium exchange determined for hCK2α at pH 6.7 (upper panel) and 8.7 (lower panel) in the free form (red) and in complex with 5,6-DBBt (blue). Peptides for which the theoretical deuteration was calculated are denoted in gray.

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