Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics


# Isomerization Mechanism of the HcRed Fluorescent Protein Chromophore

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## Supporting information

Table S1. Calculated OM2-MRCI bond distances (Å) for minima, transition states, and conical intersections.

| | OM2 | CASSCF | Δ | OM2 | CASSCF | Δ | OM2 | CASSCF | Δ | OM2 | CASSCF | Δ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *cis*-$S_0$-min($R_0$) | | | *cis*-$S_1$-plan | | | *trans*-$S_0$-min | | | *trans*-$S_1$-plan | | |
| OH-CZ | 1.246 | 1.209 | 0.037 | 1.256 | 1.220 | 0.036 | 1.244 | 1.208 | 0.036 | 1.255 | 1.220 | 0.035 |
| CZ-CE2 | 1.459 | 1.471 | -0.012 | 1.446 | 1.146 | 0.301 | 1.455 | 1.472 | -0.017 | 1.446 | 1.457 | -0.011 |
| CE2-CD2 | 1.362 | 1.357 | 0.005 | 1.379 | 1.376 | 0.003 | 1.362 | 1.353 | 0.009 | 1.380 | 1.377 | 0.003 |
| CD2-CG2 | 1.440 | 1.451 | -0.011 | 1.414 | 1.424 | -0.010 | 1.443 | 1.456 | -0.013 | 1.414 | 1.422 | -0.008 |
| CG2-CD1 | 1.443 | 1.450 | -0.007 | 1.417 | 1.418 | -0.001 | 1.447 | 1.456 | -0.009 | 1.421 | 1.422 | -0.001 |
| CD1-CE1 | 1.360 | 1.356 | 0.004 | 1.377 | 1.379 | -0.002 | 1.359 | 1.356 | 0.003 | 1.375 | 1.378 | -0.003 |
| CE1-CZ | 1.463 | 1.470 | -0.007 | 1.449 | 1.458 | -0.009 | 1.464 | 1.470 | -0.006 | 1.449 | 1.458 | -0.009 |
| CG2-CB2 | 1.395 | 1.380 | 0.015 | 1.453 | 1.468 | -0.015 | 1.386 | 1.376 | 0.010 | 1.447 | 1.466 | -0.019 |
| CB2-CA2 | 1.402 | 1.416 | -0.014 | 1.361 | 1.352 | 0.009 | 1.417 | 1.427 | -0.010 | 1.365 | 1.360 | 0.005 |
| CA2-N2 | 1.357 | 1.349 | 0.008 | 1.402 | 1.414 | -0.012 | 1.354 | 1.351 | 0.003 | 1.413 | 1.424 | -0.011 |
| N2-C1 | 1.348 | 1.320 | 0.028 | 1.327 | 1.299 | 0.028 | 1.353 | 1.319 | 0.034 | 1.323 | 1.296 | 0.027 |
| C1-N3 | 1.412 | 1.389 | 0.023 | 1.407 | 1.399 | 0.008 | 1.414 | 1.389 | 0.025 | 1.407 | 1.396 | 0.011 |
| N3-C2 | 1.386 | 1.365 | 0.021 | 1.390 | 1.358 | 0.032 | 1.385 | 1.361 | 0.024 | 1.388 | 1.357 | 0.031 |
| C2-O2 | 1.242 | 1.213 | 0.029 | 1.237 | 1.199 | 0.038 | 1.252 | 1.216 | 0.036 | 1.240 | 1.199 | 0.041 |
| C2-CA2 | 1.511 | 1.467 | 0.044 | 1.518 | 1.497 | 0.021 | 1.498 | 1.471 | 0.027 | 1.512 | 1.504 | 0.008 |
| C1-CA1 | 1.426 | 1.403 | 0.023 | 1.460 | 1.432 | 0.028 | 1.424 | 1.399 | 0.025 | 1.462 | 1.436 | 0.026 |
| CA1-N | 1.312 | 1.301 | 0.011 | 1.307 | 1.318 | -0.011 | 1.310 | 1.305 | 0.005 | 1.307 | 1.321 | -0.014 |
| N-C | 1.376 | 1.377 | -0.001 | 1.382 | 1.358 | 0.024 | 1.377 | 1.371 | 0.006 | 1.383 | 1.355 | 0.028 |
| C-O | 1.233 | 1.191 | 0.042 | 1.239 | 1.201 | 0.038 | 1.235 | 1.193 | 0.042 | 1.239 | 1.202 | 0.037 |
| | $S_1$-τ | | | *trans*-$S_{01}$-φ | | | *cis*-$S_{01}$-φ | | | $S_{01}$-τ | | |
| OH-CZ | 1.255 | 1.234 | 0.021 | 1.258 | 1.212 | 0.046 | 1.252 | 2.256 | 0.004 | 1.254 | 1.287 | -0.033 |
| CZ-CE2 | 1.450 | 1.439 | 0.011 | 1.446 | 1.464 | -0.018 | 1.453 | 1.490 | -0.037 | 1.450 | 1.387 | 0.063 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CE2-CD2 | 1.366 | 1.377 | -0.011 | 1.375 | 1.372 | 0.003 | 1.374 | 1.371 | 0.003 | 1.371 | 1.412 | -0.041 |
| CD2-CG2 | 1.432 | 1.425 | 0.007 | 1.413 | 1.423 | -0.010 | 1.414 | 1.426 | -0.012 | 1.425 | 1.376 | 0.049 |
| CG2-CD1 | 1.432 | 1.432 | 0.000 | 1.413 | 1.423 | -0.010 | 1.414 | 1.426 | -0.012 | 1.426 | 1.410 | 0.016 |
| CD1-CE1 | 1.365 | 1.370 | -0.005 | 1.375 | 1.372 | 0.003 | 1.374 | 1.371 | 0.003 | 1.371 | 1.387 | -0.016 |
| CE1-CZ | 1.451 | 1.451 | 0.000 | 1.446 | 1.462 | -0.016 | 1.453 | 1.468 | -0.015 | 1.450 | 1.430 | 0.020 |
| CG2-CB2 | 1.395 | 1.410 | -0.015 | 1.444 | 1.479 | -0.035 | 1.454 | 1.479 | -0.025 | 1.410 | 1.499 | -0.089 |
| CB2-CA2 | 1.434 | 1.458 | -0.024 | 1.375 | 1.363 | 0.012 | 1.372 | 1.360 | 0.012 | 1.414 | 1.459 | -0.045 |
| CA2-N2 | 1.302 | 1.326 | -0.024 | 1.382 | 1.373 | 0.009 | 1.372 | 1.369 | 0.003 | 1.271 | 1.290 | -0.019 |
| N2-C1 | 1.403 | 1.347 | 0.056 | 1.330 | 1.315 | 0.015 | 1.341 | 1.320 | 0.021 | 1.439 | 1.405 | 0.034 |
| C1-N3 | 1.372 | 1.369 | 0.003 | 1.401 | 1.401 | 0.000 | 1.417 | 1.406 | 0.011 | 1.340 | 1.332 | 0.008 |
| N3-C2 | 1.429 | 1.380 | 0.049 | 1.406 | 1.353 | 0.053 | 1.385 | 1.348 | 0.037 | 1.493 | 1.416 | 0.077 |
| C2-O2 | 1.225 | 1.191 | 0.034 | 1.231 | 1.201 | 0.030 | 1.238 | 1.204 | 0.034 | 1.213 | 1.157 | 0.056 |
| C2-CA2 | 1.579 | 1.508 | 0.071 | 1.514 | 1.506 | 0.008 | 1.522 | 1.504 | 0.018 | 1.687 | 1.579 | 0.108 |
| C1-CA1 | 1.433 | 1.423 | 0.010 | 1.453 | 1.400 | 0.053 | 1.434 | 1.393 | 0.041 | 1.427 | 1.469 | -0.042 |
| CA1-N | 1.302 | 1.291 | 0.011 | 1.295 | 1.322 | -0.027 | 1.310 | 1.330 | -0.020 | 1.317 | 1.267 | 0.050 |
| N-C | 1.398 | 1.403 | -0.005 | 1.401 | 1.351 | 0.050 | 1.385 | 1.341 | 0.044 | 1.389 | 1.448 | -0.059 |
| C-O | 1.229 | 1.183 | 0.046 | 1.229 | 1.203 | 0.026 | 1.234 | 1.208 | 0.026 | 1.234 | 1.168 | 0.066 |

Table S2. Relevant important dihedral angles (º) and hydrogen bond distances (Å) of *cis* and *trans* conformers and of the transition state of HcRed (ground state, model B) at the SCC-DFTB/MM level.

| conformations | *cis* | TS | *trans* |
|---|---|---|---|
| Dihedral angle | | | |
| N2_CA2_CB2_CG2 | 4.8 | 136.8 | 173.4 |
| CA2_CB2_CG2_CD1 | 4.4 | -83.7 | -157.0 |
| Distance | | | |
| O_NE2(Gln107) | 3.192 | 3.104 | 3.301 |
| O2_NH2(Arg93) | 2.621 | 2.655 | 2.656 |
| OH_OG(Ser144) | 2.783 | 2.737 | |
| N2_OE2(Glu214) | 3.192 | 2.871 | 2.955 |
| N2_NE2(Gln40) | 2.772 | 2.791 | 2.774 |
| OH_ND2(Asn159) | | | 2.977 |

Table S3. QM energies (a.u.), MM energies (a.u.), total QM/MM energies (a.u.), and relative energies (kcal/mol) for the *cis*- and *trans*-conformers and the transition state in model B of HcRed: DFT(B3LYP/TZVP)/MM results for snapshots 1-4.

| snapshot | | $E_{(QM,MM)}$ (a.u.) | $\Delta E_Q$ (kcal/mol) | $E_{(MM,QM)}$ (a.u.) | $\Delta E_M$ (kcal/mol) | $E_{total}$ (a.u.) | $\Delta E_t$ (kcal/mol) |
|---|---|---|---|---|---|---|---|
| 1 | *cis* | -1480.61278 | 0.0 | -55.06988 | 0.0 | -1535.68265 | 0.0 |
| | $TS_{\_HT}$ | -1480.51851 | 59.2 | -55.06142 | 5.3 | -1535.57993 | 64.5 |
| | *trans* | -1480.59630 | 10.3 | -55.06777 | 1.3 | -1535.66407 | 11.7 |
| 2 | *cis* | -1480.60572 | 0.0 | -56.71797 | 0.0 | -1537.32369 | 0.0 |
| | $TS_{\_HT}$ | -1480.48591 | 75.2 | -56.74158 | -14.8 | -1537.22751 | 60.4 |
| | *trans* | -1480.55969 | 28.9 | -56.75081 | -20.6 | -1537.31051 | 8.3 |
| 3 | *cis* | -1480.60259 | 0.0 | -56.65632 | 0.0 | -1537.25892 | 0.0 |
| | $TS_{\_HT}$ | -1480.50415 | 61.8 | -56.65938 | -1.9 | -1537.16353 | 59.9 |
| | *trans* | -1480.58119 | 13.4 | -56.6677 | -7.1 | -1537.24884 | 6.3 |
| 4 | *cis* | -1480.62035 | 0.0 | -56.96162 | 0.0 | -1537.58196 | 0.0 |
| | $TS_{\_HT}$ | -1480.52208 | 61.7 | -56.97113 | -6.0 | -1537.49320 | 55.7 |
| | *trans* | -1480.58861 | 19.9 | -56.97643 | -9.3 | -1537.56504 | 10.6 |

Table S4. Relative energies (kcal/mol) of the *cis* and *trans* conformations and the transition state of HcRed of model A from SCC-DFTB/MM geometry optimizations and from single-point DFT(B3LYP/TZVP)/MM calculations at SCC-DFTB/MM optimized geometries for snapshots 1-4.

| Snapshots | *Cis* | $TS_{\_HT}$ | *trans* |
|---|---|---|---|
| SCC-DFTB/MM level | | | |
| 1 | 0.0 | 41.6 | -0.5 |
| 2 | 0.0 | 41.8 | -2.0 |
| 3 | 0.0 | 39.1 | -5.1 |
| 4 | 0.0 | 42.8 | -3.2 |
| B3LYP/MM// SCC-DFTB/MM level | | | |
| 1 | 0.0 | 47.5 | -1.1 |
| 2 | 0.0 | 42.9 | -3.6 |
| 3 | 0.0 | 41.6 | -2.0 |
| 4 | 0.0 | 43.7 | -3.1 |

Table S5. QM energies (a.u.), MM energies (a.u.), total QM/MM energies (a.u.), and relative energies (kcal/mol) for the *cis*- and *trans*-conformers and the transition state in model A of HcRed: DFT(B3LYP/TZVP)/MM results for snapshots 1-4.

| snapshot | | $E_{(QM,MM)}$ (a.u.) | $\Delta E_Q$ (kcal/mol) | $E_{(MM,QM)}$ (a.u.) | $\Delta E_M$ (kcal/mol) | $E_{total}$ (a.u.) | $\Delta E_t$ (kcal/mol) |
|---|---|---|---|---|---|---|---|
| 1 | *cis* | -1480.69689 | 0.0 | -56.14492 | 0.0 | -1536.84181 | 0.0 |
| | $TS_{\_HT}$ | -1480.62647 | 44.2 | -56.13964 | 3.3 | -1536.76611 | 47.5 |
| | *trans* | -1480.70326 | -4.0 | -56.14023 | 2.9 | -1536.84349 | -1.1 |
| 2 | *cis* | -1480.71610 | 0.0 | -54.96923 | 0.0 | -1535.68533 | 0.0 |
| | $TS_{\_HT}$ | -1480.65079 | 41.0 | -54.96623 | 1.9 | -1535.61702 | 42.9 |
| | *trans* | -1480.70997 | 3.8 | -54.98109 | -7.4 | -1535.69106 | -3.6 |
| 3 | *cis* | -1480.68720 | 0.0 | -57.10091 | 0.0 | -1537.78811 | 0.0 |
| | $TS_{\_HT}$ | -1480.62288 | 40.4 | -57.09894 | 1.2 | -1537.72182 | 41.6 |
| | *trans* | -1480.68609 | 0.7 | -57.10514 | -2.7 | -1537.79124 | -2.0 |
| 4 | *cis* | -1480.70062 | 0.0 | -56.14165 | 0.0 | -1536.84226 | 0.0 |
| | $TS_{\_HT}$ | -1480.63448 | 41.5 | -56.13812 | 2.2 | -1536.77259 | 43.7 |
| | *trans* | -1480.69271 | 5.0 | -56.15447 | -8.0 | -1536.84718 | -3.1 |

Table S6. Relative energies (kcal/mol) of the *cis* and *trans* conformations and the transition states of HcRed of model C from SCC-DFTB/MM geometry optimizations and from single-point DFT(B3LYP/TZVP)/MM calculations at SCC-DFTB/MM optimized geometries for snapshots 1-4.

| Snapshots | *cis* | $TS_{\_HT}$ | *trans* |
|---|---|---|---|
| SCC-DFTB/MM level | | | |
| 1 | 0.0 | 44.8 | 12.7 |
| 2 | 0.0 | 53.5 | 12.0 |
| 3 | 0.0 | 50.2 | 8.7 |
| 4 | 0.0 | 50.0 | 15.4 |
| B3LYP/MM// SCC-DFTB/MM level | | | |
| 1 | 0.0 | 50.2 | 19.0 |
| 2 | 0.0 | 57.4 | 11.7 |
| 3 | 0.0 | 64.9 | 15.3 |
| 4 | 0.0 | 63.1 | 20.9 |

Table S7. QM energies (a.u.), MM energies (a.u.), total QM/MM energies (a.u.), and relative energies (kcal/mol) for the *cis*- and *trans*-conformers and the transition state in model C of HcRed: DFT(B3LYP/TZVP)/MM results for snapshots 1-4.

| snapshot | | $E_{(QM,MM)}$ (a.u.) | $\Delta E_Q$ (kcal/mol) | $E_{(MM,QM)}$ (a.u.) | $\Delta E_M$ (kcal/mol) | $E_{total}$ (a.u.) | $\Delta E_t$ (kcal/mol) |
|---|---|---|---|---|---|---|---|
| 1 | *cis* | -1480.63291 | 0.0 | -57.02583 | 0.0 | -1537.65874 | 0.0 |
| | $TS_{\_HT}$ | -1480.54215 | 57.0 | -57.03661 | -6.8 | -1537.57875 | 50.2 |
| | *trans* | -1480.59310 | 25.0 | -57.03539 | -6.0 | -1537.62849 | 19.0 |
| 2 | *cis* | -1480.58778 | 0.0 | -56.80985 | 0.0 | -1537.39763 | 0.0 |
| | $TS_{\_HT}$ | -1480.50309 | 53.1 | -56.81213 | -1.4 | -1537.30614 | 57.4 |
| | *trans* | -1480.56690 | 13.1 | -56.80304 | 4.3 | -1537.37903 | 11.7 |
| 3 | *cis* | -1480.60365 | 0.0 | -56.66452 | 0.0 | -1537.26817 | 0.0 |
| | $TS_{\_HT}$ | -1480.49972 | 65.2 | -56.66496 | -0.3 | -1537.16468 | 64.9 |
| | *trans* | -1480.55412 | 31.1 | -56.68962 | -15.8 | -1537.24374 | 15.3 |
| 4 | *cis* | -1480.61529 | 0.0 | -56.45431 | 0.0 | -1537.06960 | 0.0 |
| | $TS_{\_HT}$ | -1480.52690 | 55.5 | -56.44212 | 7.7 | -1536.96902 | 63.1 |
| | *trans* | -1480.56534 | 31.3 | -56.47095 | -10.4 | -1537.03629 | 20.9 |

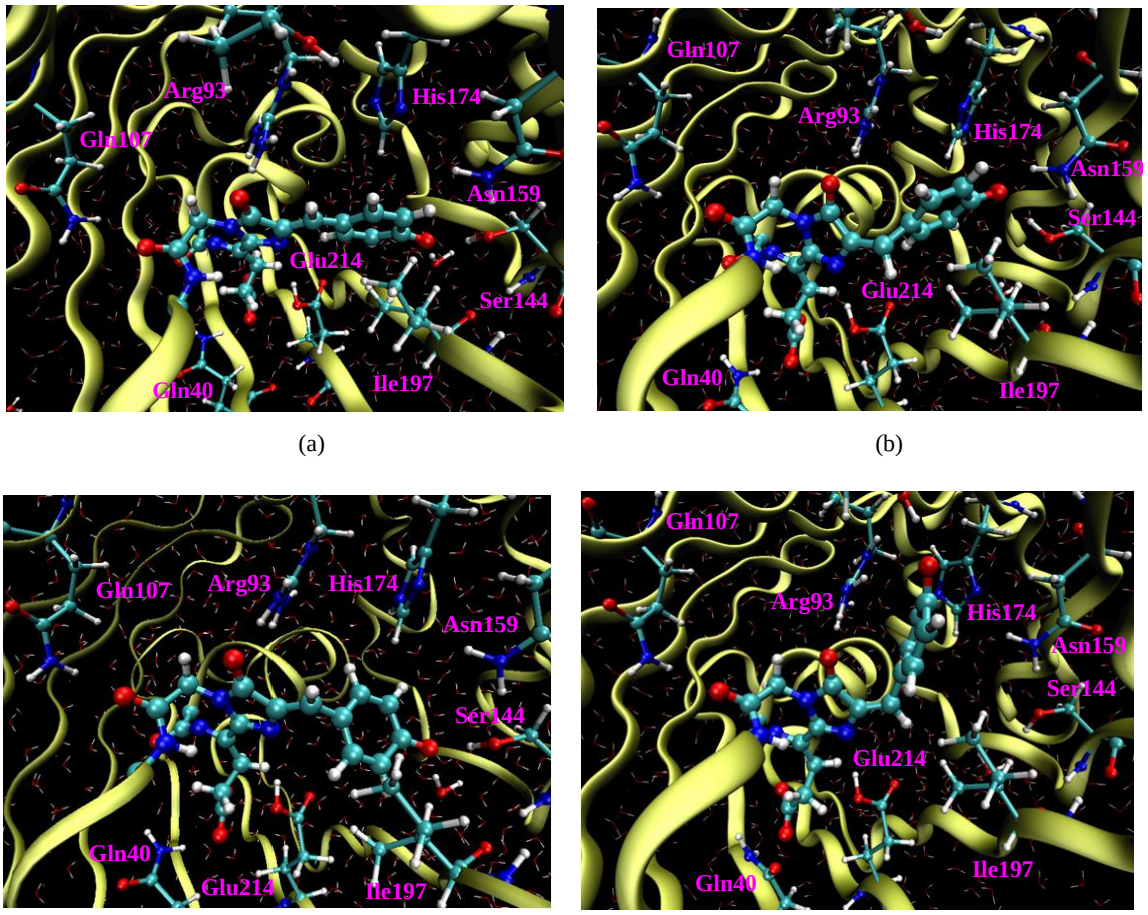


Figure S1. Optimized OM2-MRCI/MM structures for snapshot 1: (a) cis-chromophore of HcRed in the first excited state; (b) conical intersection for *cis-trans* isomerization via HT motion; (c) conical intersection reached from *cis*-conformer via φ rotation; (d) conical intersection reached from *trans*-conformer via φ rotation.