**Supplementary file 1a.** Amino-acid sequence settings in the MC molecular simulation. The residues used to define the dipole of the chromophoric groups are indicated.

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| GFP | kept rigid | MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV**N**RIELKGIDFKEDGNILGHKLEYNY**N**SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAA |
| flexible | GITLGMDELYKSGLRSELNFEFPGASEIHMSEP |
| mGBP2 | kept rigid | MCLIENTEAQLVINQEALRILSAITQPVVVVAIVGLYRTGKSYLMNKLAGKRTGFSLGSTVQSHTKGIWMWCVPHPKKAGQTLVLLDTEGLEDVEKGDNQNDCWIFALAVLLSSTFIYNSIGTINQQAMDQLHYVTELTDLIKSKSSPDQSGVDDSANFVGFFPTFVWTLRDFSLELEVNGKPVTSDEYLEHSLTLKKGADKKTKSFNEPRLCIRKFFPKRKCFIFDRPAQRKQLSKLETLREEELCGEFVEQVAEFTSYILSYSSVKTLCGGIIVNGPRLKSLVQTYVGAISNGSLPCMESAVLTLAQIENSAAVQKAITHYEEQMNQKIQMPTETLQELLDLHRPIESEAIEVFLKNSFKDVDQKFQTELGNLLVAKRDAFIKKNMDVSSARCSDLLEDIFGPLEEEVKLGTFSKPGGYYLFLQMRQELEKKYNQAPGKGLQAEAMLKNYFDSKADVVETLLQTDQSLTEAAKEVEEERTKAEAAEAANRELEKKQKEFELMMQQKEKSYQEHVKKLTEKMKDEQKQLLAEQENIIAAKLREQEKFLKEGFENESKKLIREIDTLKQNKSSGKCTIL |

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| mCherry | kept rigid | MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFI**Y**KVKLRGTNFPSDGPVMQKKTMGW**E**ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIV |
| flexible | EQYERAEGRHSTGGMDELYKEFPGASEIHMSEP |
| mGBP2 | kept rigid | MCLIENTEAQLVINQEALRILSAITQPVVVVAIVGLYRTGKSYLMNKLAGKRTGFSLGSTVQSHTKGIWMWCVPHPKKAGQTLVLLDTEGLEDVEKGDNQNDCWIFALAVLLSSTFIYNSIGTINQQAMDQLHYVTELTDLIKSKSSPDQSGVDDSANFVGFFPTFVWTLRDFSLELEVNGKPVTSDEYLEHSLTLKKGADKKTKSFNEPRLCIRKFFPKRKCFIFDRPAQRKQLSKLETLREEELCGEFVEQVAEFTSYILSYSSVKTLCGGIIVNGPRLKSLVQTYVGAISNGSLPCMESAVLTLAQIENSAAVQKAITHYEEQMNQKIQMPTETLQELLDLHRPIESEAIEVFLKNSFKDVDQKFQTELGNLLVAKRDAFIKKNMDVSSARCSDLLEDIFGPLEEEVKLGTFSKPGGYYLFLQMRQELEKKYNQAPGKGLQAEAMLKNYFDSKADVVETLLQTDQSLTEAAKEVEEERTKAEAAEAANRELEKKQKEFELMMQQKEKSYQEHVKKLTEKMKDEQKQLLAEQENIIAAKLREQEKFLKEGFENESKKLIREIDTLKQNKSSGKCTIL |

**Supplementary file 1b.** Calculations of donor-acceptor distances (*Rsim*) and orientation factors (2) from each sampled conformation from MC molecular simulation of G-mGBP2/mCh-mGBP2 dimer in steps. See Experimental procedures and Figure S7 for details.

|  |  |  |
| --- | --- | --- |
|  | Donor (GFP) | Acceptor (mCherry) |
| Coordinates of the two chosen C-atoms | and | and |
| Distance between the two C-atoms |  |  |
| Unit vector connecting the two C-atoms |  |  |
| Coordinates of the middle point of the connecting vector |  |  |
| Calculation of donor-acceptor distance and orientation factor |  | |