CONTRIBUTION OF FLUOROPHORE DYNAMICS AND SOLVATION TO RESONANT ENERGY TRANSFER IN PROTEIN-DNA COMPLEXES: A MOLECULAR-DYNAMICS STUDY

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Supporting Material

Table S1: List of systems simulated in this work

System	Trajectory duration, ns
DNA duplex in water	W/O ATTO dyes: **46** W/ ATTO dyes: **58**
DNA- Cre synapsis (5CRX) in water	W/O ATTO dyes: **49** W/ ATTO dyes parallel: **57** W/ ATTO dyes antiparallel: **58**
DNA Holiday junction without Cre in water	W/O ATTO dyes: **60** W/ ATTO dyes scenario (I) : **56** W/ ATTO dyes scenario (II): **51**
DNA-Cre Holiday junction (3CRX) in water	W/O ATTO dyes: **45** W/ ATTO dyes scenario (I) : **54** W/ ATTO dyes scenario (II): **57**
DNA-Cre Holiday junction (3CRX) in water:glycerol	W/ ATTO dyes scenario (II): **200**
Controls- Tethered dyes in water	d= 2 nm: **30** d= 3 nm: **30** d= 4 nm: **30** d= 5 nm: **30** d= 7.3 nm: **30**
Controls- Tethered dyes in water:glycerol	d= 2 nm: **100** d= 4 nm: **110** d= 7.3 nm: **100**

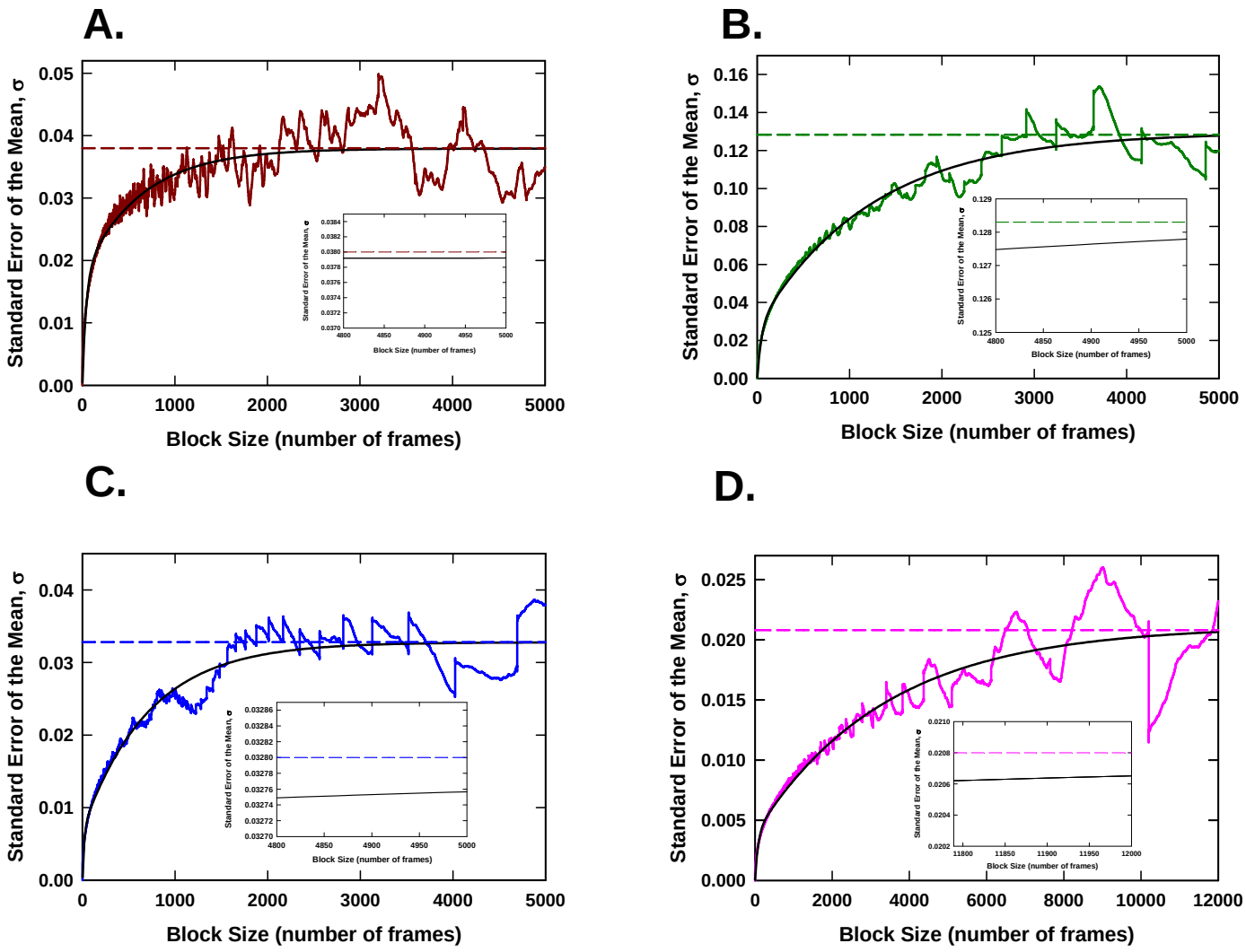

Figure S1. Block-averaging analysis of MD trajectories for convergence and statistical noise. The solid black lines show a mono- or bi-exponential fit to the apparent standard error, σ, in $\langle\kappa^2(t)\rangle_T$ as a function of data-block size. The dashed line gives the asymptotic value of the exponential fit, σ*, which corresponds to an estimated lower bound for the standard error in $\langle\kappa^2(t)\rangle_T$ over the full trajectory. Corresponding systems are: (A.) DNA duplex (system (ii.)), (B.) antiparallel Cre-DNA synaptic complex (system (iii.)), (C.) and (D.) Cre-DNA Holliday-junction intermediate (system (iv.)) in water; and glycerol:water, respectively. Insets show the respective approaches of the fitted dependencies to their asymptotic values in the limit of large block size. The fitted values for maximum block size and corresponding asymptotic values are similar at the 1% level; although this suggests sufficient equilibration of $\langle\kappa^2(t)\rangle_T$ to support the conclusions of this study, these trajectories are likely not long enough to supply an upper bound for σ*.

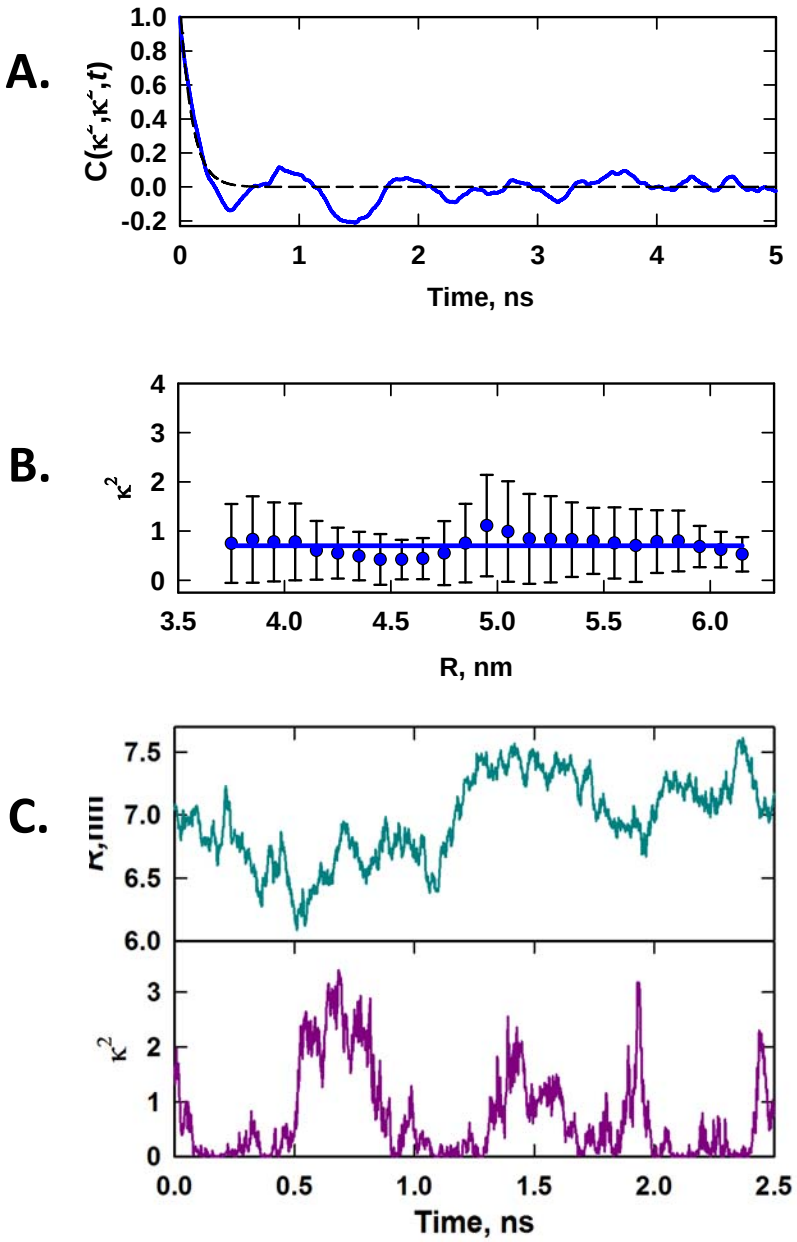

Figure S2. Dynamics of tethered ATTO dyes with *d* = 5 nm; total trajectory duration was 30 ns. **(A.)** The autocorrelation function for κ^2, $C(\kappa^2,\kappa^2;\tau) = <\kappa^2(t)\kappa^2(t+\tau)>_T$ (solid blue line) with a single-exponential fit (dashed black line). The decay time τ of the fit is 100 ps. **(B.)** Binned scatter plot of κ^2 as a function of *R* for the tethered dyes. The average value of κ^2 is 0.65, shown as a solid blue line. **(C.)** Time-dependent fluctuations of the orientation factor, κ^2 and center-of-mass distance between the dyes, *R*.

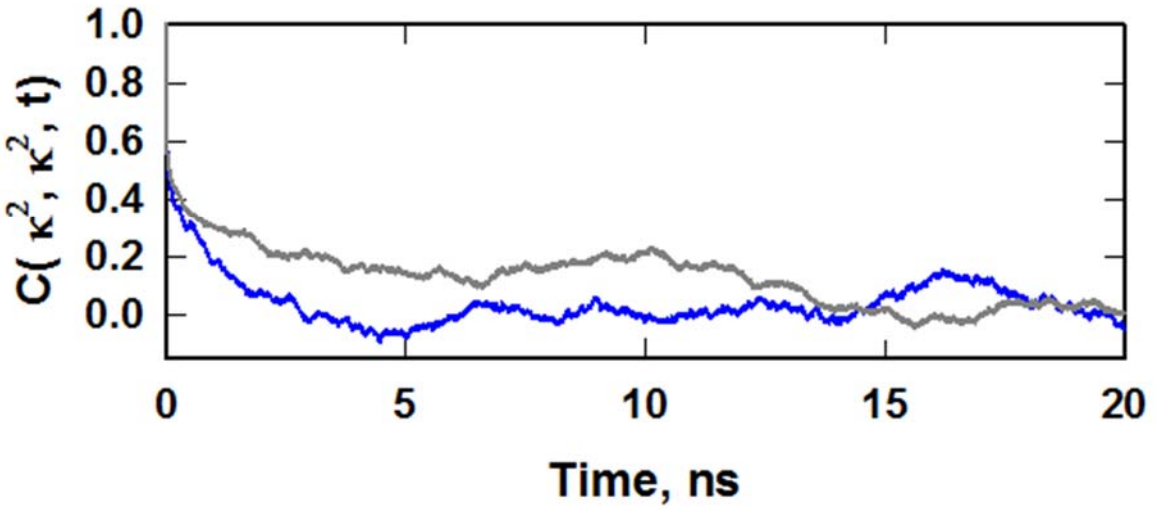

Figure S3. Dynamics of ATTO dyes conjugated to a Cre-DNA holliday junction complex. The autocorrelation function for κ^2, $C(\kappa^2,\kappa^2;\tau) = <\kappa^2(t)\kappa^2(t+\tau)>_T$ is shown for system (iv.) in water (blue) and glycerol:water (grey). We estimate decay times of 1 ns (aqueous) and 9 ns (glycerol:water). MD trajectories for the Cre-HJ complex in water and glycerol:water were carried out for times that are more than 20 times the respective autocorrelation-decay times for κ^2.

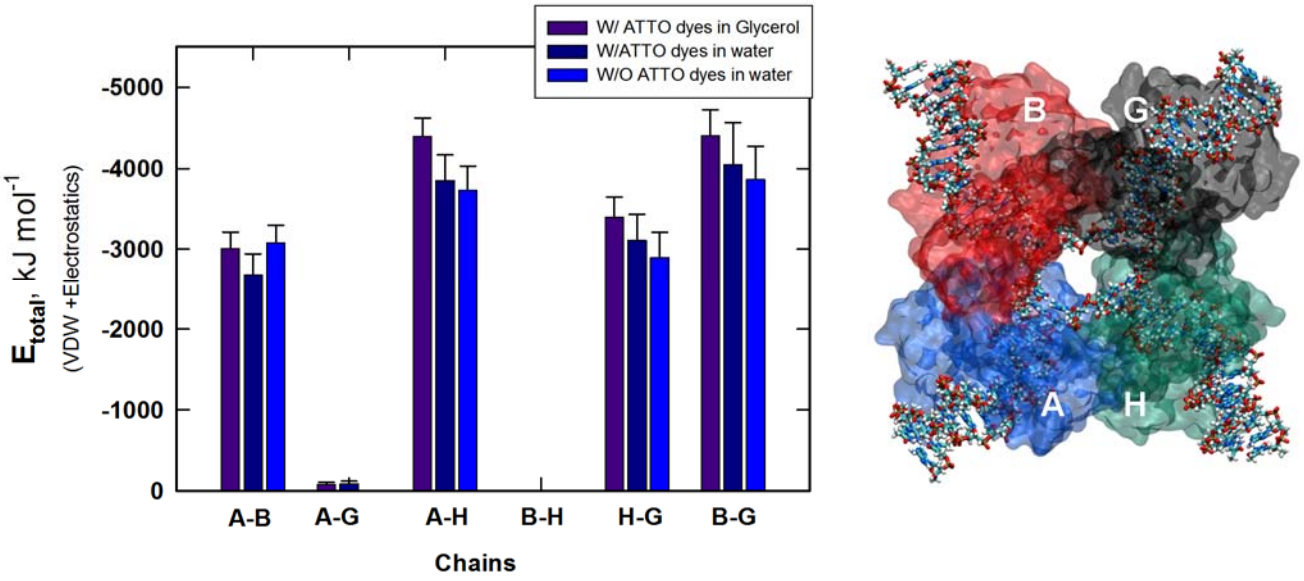

Figure S4. Stabilization energy of the 3CRX Cre-HJ complex. The sum of van der Waals and electrostatic components of the potential energy of interaction between Cre monomers, labeled A, B, G, and H. All energetic contributions from DNA binding, as well as DNA-DNA interactions, are excluded. Note that incorporation of ATTO dyes does not affect the stability of the complex in water. There is a small, but measurable, stabilization of protein-protein interactions in glycerol-water mixture.

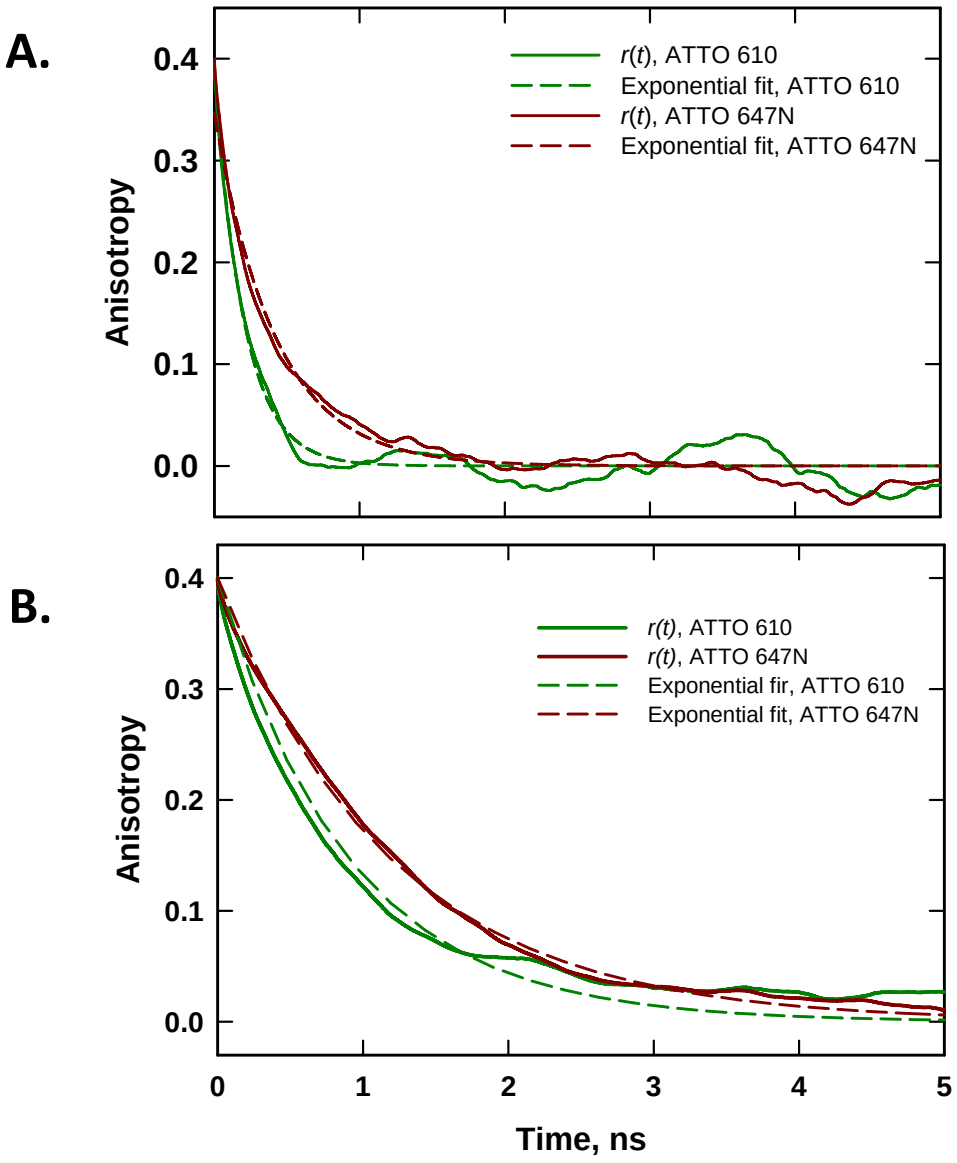

Figure S5. Time-dependent anisotropy, $r(t)$, of individual ATTO dyes**. (A.)** Simulations in aqueous-solvent conditions. Single-exponential fits yielded correlation times of 400 and 640 ps for ATTO 610 and ATTO 647N, respectively. The smaller value attributed to ATTO 610 is expected due to the smaller dimensions of the fluorophore. **(B)** Simulations in water:glycerol solvent yielded correlation times of 1.2 and 1.7 ns for ATTO 610 and ATTO 647N, respectively. The relative time constants for water:glycerol mixtures and aqueous solvent are quantitatively consistent with the increment in bulk solvent viscosity.

Appendix: Topology Files for ATTO 647N and ATTO 610 (CHARMM Format)

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!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!!!! ATTO Dyes Topology file By: Massa Shoura and Udy Ranatunga 2013!!!!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!!!!!!!! ATTO 647N Dye molecule topology!!!!!!!!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

RESI A647         +1.00 !

!N and adjacent C
GROUP
ATOM C34  CA      0.000 !
GROUP
ATOM C41  CA     -0.115 !
ATOM H25  HP      0.115 !
GROUP
ATOM C40  CA      0.000 !
GROUP
ATOM C35  CA     -0.115 !
ATOM H41  HP      0.115 !
GROUP                   !
ATOM C39  CT2    -0.18  !
ATOM H27  HA      0.09  !
ATOM H26  HA      0.09  !
GROUP                   !
ATOM C38  CT2    -0.18  !
ATOM H29  HA      0.09  !
ATOM H28  HA      0.09  !

!delocalized N group
GROUP
! 1st N and its neighbors
ATOM N4   N3R    -0.506 !         ESP charge optimized
ATOM C37  CT      0.558 !         ESP charge optimized
ATOM C46  CT2     0.147 !         ESP charge optimized
ATOM H40  HA      0.09  !
ATOM H42  HA      0.09  !
ATOM C36  CA      0.434 !         ESP charge optimized
! 2nd N+ and its neighbors
ATOM N5   NRA    -0.067 !         ESP charge optimized
ATOM C27  CT2     0.030 !         ESP charge optimized
ATOM H52  HA      0.09  !
ATOM H53  HA      0.09  !
ATOM C43  CA      0.077 !         ESP charge optimized
ATOM C28  CT2    -0.223 !         ESP charge optimized
ATOM H54  HA      0.09  !
ATOM H55  HA      0.09  !

GROUP                   !
ATOM C49  CT3    -0.27  !
ATOM H31  HA      0.09  !
ATOM H32  HA      0.09  !
```

```
ATOM H33  HA      0.09  !
GROUP                   !
ATOM C48  CT3    -0.27  !
ATOM H34  HA      0.09  !
ATOM H35  HA      0.09  !
ATOM H36  HA      0.09  !
GROUP                   !
ATOM C47  CT3    -0.27  !
ATOM H37  HA      0.09  !
ATOM H38  HA      0.09  !
ATOM H39  HA      0.09  !

!RING
GROUP                   !
ATOM C26  CE1    -0.15  !
ATOM C25  CE1    -0.15  !
ATOM H51  HE1     0.15  !
ATOM H50  HE1     0.15  !

!RING
GROUP                   !
ATOM C30  CT2    -0.18  !
ATOM H59  HA      0.09  !
ATOM H58  HA      0.09  !
GROUP                   !
ATOM C29 CT2     -0.18  !
ATOM H56  HA      0.09  !
ATOM H57  HA      0.09  !

!RING
GROUP                   !
ATOM C23  CA     -0.115 !
ATOM H49  HP      0.115 !
GROUP                   !
ATOM C24  CA      0.000 !
GROUP                   !
ATOM C31  CA      0.000 !
!RING
GROUP                   !
ATOM C42  CA      0.000 !
GROUP                   !
ATOM C33  CT      0.000 !
GROUP                   !
ATOM C32  CA      0.000 !
GROUP                   !
ATOM C22  CA      0.000 !
GROUP                   !
ATOM C21  CA      0.000 !
GROUP                   !
ATOM C44  CT3    -0.27  !
ATOM H46  HA      0.09  !
ATOM H47  HA      0.09  !
ATOM H48  HA      0.09  !
GROUP                   !
```

```
ATOM C45  CT3    -0.27  !
ATOM H43  HA      0.09  !
ATOM H44  HA      0.09  !
ATOM H45  HA      0.09  !

!RING
GROUP                   !
ATOM C16  CA     -0.115 !
ATOM H20  HP      0.115 !
GROUP                   !
ATOM C17  CA     -0.115 !
ATOM H21  HP      0.115 !
GROUP                   !
ATOM C18  CA     -0.115 !
ATOM H22  HP      0.115 !
GROUP                   !
ATOM C19  CA     -0.115 !
ATOM H23  HP      0.115 !
GROUP                   !
ATOM C15  CA      0.000 !
GROUP                   !
ATOM C20  CA      0.000 !
GROUP                   !
ATOM C14  CC      0.55  !
ATOM O3   O      -0.55  !
GROUP                   !
ATOM N3   N      -0.29  ! -PRO
!ATOM HN3  H      0.31  !  |
ATOM C50    CT3        0.02  !  |
ATOM H60    HA         0.09  !  |
ATOM H61    HA         0.09  !  |
ATOM H62  HA           0.09  !  /
GROUP
ATOM C13  CT2    -0.18  !
ATOM H18  HA      0.09  !
ATOM H19  HA      0.09  !

! BUTANAL PENDANT
GROUP                   !
ATOM C12  CT2    -0.18  !
ATOM H16  HA      0.09  !
ATOM H17  HA      0.09  !
GROUP                   !
ATOM C11  CT2    -0.18  !
ATOM H24  HA      0.09  !
ATOM H15  HA      0.09  !
GROUP                   !
ATOM C10  CC      0.55  !
ATOM O2   O      -0.55  !

!LINKER
GROUP                   !
ATOM N2   NH2    -0.47  ! -ALAD residue
ATOM HN2  H       0.31  !  |
```

```
ATOM C9   CT2    -0.02  !  |
ATOM H14  HA      0.09  !  |
ATOM H13  HA      0.09  ! _/
GROUP                   !
ATOM C8   CT2    -0.18  !
ATOM H11  HA      0.09  !
ATOM H12  HA      0.09  !
GROUP                   !
ATOM C7   CT2    -0.18  !
ATOM H9   HA      0.09  !
ATOM H10  HA      0.09  !
GROUP                   !
ATOM C6   CT2    -0.18  !
ATOM H7   HA      0.09  !
ATOM H8   HA      0.09  !
GROUP                   !
ATOM C5   CT2    -0.18  !
ATOM H5   HA      0.09  !
ATOM H6   HA      0.09  !
GROUP                   !
ATOM C4   CT2    -0.01  ! -ALAD residue
ATOM H3   HA      0.09  !  |
ATOM H4   HA      0.09  !  |
ATOM N1   NH2    -0.47  !  |
ATOM HN1  H       0.31  ! _/
GROUP                   !
ATOM C3   CC      0.55  !
ATOM O1   O      -0.55  !
GROUP                   !
ATOM C1  CE1     -0.15  ! -PRPE residues
ATOM C2  CE1     -0.15  !  |
ATOM H1  HE1      0.15  !  |
ATOM H2  HE1      0.15  ! _/

DOUBLE C1 C2                                            !
DOUBLE C3 O1                                            !
DOUBLE C14 O3                                           !
DOUBLE C10 O2                                           !

BOND C1  H1                                             !
BOND C2  H2                                             !
BOND C2  C3  C3  N1  N1  HN1 N1  C4  C4  H3 C4 H4       !
BOND C4  C5  C5  H5  C5  H6  C5  C6  C6  H7 C6 H8       !
!BOND C7  C6  C7  H9  C7  H10 C7  C8  H11 C8 H12      !
!BOND C8  C9  C9  N2  N2  HN2 N2  C10                 !
BOND C6  C7  C7  C8  C8  C9  C9  N2  N2  C10          !
BOND C7  H9  C7  H10
BOND N2  HN2 C8  H11 C8  H12 C9  H13 C9  H14          !
BOND C10 C11 C11 H24 C11 H15 C11 C12 C12 H16 C12 H17     !
BOND C12 C13 C13 H18 C13 H19 C13 N3  N3  C50 N3 C14   !
BOND C50 H60 C50 H61 C50 H62                                    !
BOND C14 C15 C15 C16 C16 H20 C16 C17 C17 H21 C17 C18     !
BOND C18 H22 C18 C19 C19 H23 C19 C20 C20 C15 C20 C21     !
BOND C21 C22 C22 C23 C23 H49 C23 C24 C24 C43 C43 C31     !
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BOND C31 C32 C32 C22   C21 C42 C42 C34 C34 C33 C33 C32    !
BOND C33 C45 C33 C44
                      !
BOND C45 H43 C45 H44 C45 H45 C44 H46 C44 H47 C44 H48     !
BOND C24 C25 C25 H50 C25 C26 C26 H51 C26 C27                    !
BOND C27 H52 C27 H53 C27 N5  N5  C28 N5  C43          !
BOND C28 H54 C28 H55 C28 C29
      !
BOND C29 H56 C29 H57 C29 C30
      !
BOND C30 H58 C30 H59 C30 C31
      !
BOND C47 H37 C47 H38 C47 H39 C47 C46
BOND C46 H42 C46 H40 C46 N4  N4  C37
BOND C48 H34 C48 H35 C48 H36 C48 C37
BOND C49 H31 C49 H32 C49 H33 C49 C37
BOND C38 H28 C38 H29 C37 C38 C38 C39
BOND C39 H26 C39 H27 C39 C40 C40 C36
BOND C36 N4  C36 C35 C35 H41 C35 C34
BOND C41 C40 C41 C42 C41 H25


PRES LNK2        -0.00   ! patch for linking ATTO 647N to THY C5M
                                                !
GROUP                          !
ATOM 2N1   NN2B   -0.34  !
ATOM 2C6   CN3     0.17  !
ATOM 2H6   HN3     0.17  !
ATOM 2C2   CN1T    0.51  !
ATOM 2O2   ON1    -0.41  !
ATOM 2N3   NN2U   -0.46  !
ATOM 2H3   HN2     0.36  !
ATOM 2C4   CN1     0.50  !
ATOM 2O4   ON1    -0.45  !
ATOM 2C5   CN3T   -0.15  !
ATOM 2C5M  CN9    -0.04  !
ATOM 2H52  HN9     0.07  !
ATOM 2H53  HN9     0.07  !
BOND 1C1   2C5M
DELETE ATOM 2H51

!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!!!!!!!! ATTO 610 Dye molecule topology!!!!!!!!!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

RESI A610        +1.00  ! topology of ATTO-610 dye
                        ! Massa Shoura Oct 21st 2011
                                              ! updated, June 27, 2012 MS
!N and adjacent C
GROUP
ATOM C19  CT3    -0.161 ! -0.171 ! ESP charge optimized
ATOM H13  HA      0.09  !
ATOM H14  HA      0.09  !
ATOM H15  HA      0.09  !
```

```
ATOM C18  CT3    -0.179 ! -0.189 ! ESP charge optimized
ATOM H10  HA      0.09  !
ATOM H11  HA      0.09  !
ATOM H12  HA      0.09  !
ATOM N1   NRA    -0.090 ! -0.100 ! ESP charge optimized
ATOM C15  CA      0.497 !  0.487 ! ESP charge optimized
ATOM C3   CA      0.333 !  0.323 ! ESP charge optimized
ATOM N2   N3R    -0.044 ! -0.054 ! ESP charge optimized
ATOM C7   CT2    -0.060 ! -0.070 ! ESP charge optimized
ATOM H39  HA      0.09  !
ATOM H5   HA      0.09  !
ATOM C20  CT2    -0.206 ! -0.23  ! ESP charge optimized
ATOM H16  HA      0.09  !
ATOM H17  HA      0.09  !

GROUP                   !
ATOM C16  CA     -0.115 !
ATOM H8   HP      0.115 !
GROUP                   !
ATOM C17  CA     -0.115 !
ATOM H9   HP      0.115 !
GROUP                   !
ATOM C14  CA     -0.115 !
ATOM H7   HP      0.115 !
GROUP                   !
ATOM C12  CA      0.000 !
GROUP                   !
ATOM C13  CA      0.000 !

! RING 2
GROUP                   !
ATOM C34  CT3    -0.27  !
ATOM H44  HA      0.09  !
ATOM H45  HA      0.09  !
ATOM H46  HA      0.09  !
GROUP                   !
ATOM C33  CT3    -0.27  !
ATOM H41  HA      0.09  !
ATOM H42  HA      0.09  !
ATOM H43  HA      0.09  !
GROUP                   !
ATOM C10  CT      0.00  !
GROUP                   !
ATOM C11  CA     -0.115 !
ATOM H6   HP      0.115 !
GROUP                   !
ATOM C8   CA      0.000 !
GROUP                   !
ATOM C9   CA      0.000 !

!RING 3
GROUP                   !
ATOM C1 CA       -0.115 !
ATOM H1 HP        0.115 !
```

```
GROUP                   !
ATOM C2 CA       -0.115 !
ATOM H2 HP        0.115 !
GROUP                   !
ATOM C4 CA        0.000 !

!RING 4
GROUP
ATOM C5   CT2    -0.18  !
ATOM H3   HA      0.09  !
ATOM H40  HA      0.09  !
GROUP                   !
ATOM C6   CT2    -0.18  !
ATOM H4   HA      0.09  !
ATOM H38  HA      0.09  !

! BUTANAL PENDANT
GROUP                   !
ATOM C21  CT2    -0.18  !
ATOM H18  HA      0.09  !
ATOM H19  HA      0.09  !
GROUP                   !
ATOM C22  CT2    -0.18  !
ATOM H20  HA      0.09  !
ATOM H21  HA      0.09  !
GROUP                   !
ATOM C23  CC      0.55  !
ATOM O1   O      -0.55  !

! LINKER
GROUP                   !
ATOM N3   NH2    -0.47  ! -ALAD residue
ATOM H22  H       0.31  !  |
ATOM C24  CT2    -0.02  !  |
ATOM H23  HA      0.09  !  |
ATOM H24  HA      0.09  ! _/
GROUP                   !
ATOM C25  CT2    -0.18  !
ATOM H25  HA      0.09  !
ATOM H26  HA      0.09  !
GROUP                   !
ATOM C26  CT2    -0.18  !
ATOM H27  HA      0.09  !
ATOM H28  HA      0.09  !
GROUP                   !
ATOM C27  CT2    -0.18  !
ATOM H29  HA      0.09  !
ATOM H30  HA      0.09  !
GROUP                   !
ATOM C28  CT2    -0.18  !
ATOM H31  HA      0.09  !
ATOM H32  HA      0.09  !
GROUP                   !
ATOM C29  CT2    -0.01  ! -ALAD residue
```

```
ATOM H33  HA       0.09  !  |
ATOM H34  HA       0.09  !  |
ATOM N4   NH2     -0.47  !  |
ATOM H35  H        0.31  ! _/
GROUP                    !
ATOM C30  CC       0.55  !
ATOM O2   O       -0.55  !
GROUP                    !
ATOM C31  CE1     -0.15  ! -PRPE residues
ATOM C32  CE1     -0.15  !  |
ATOM H36  HE1      0.15  !  |
ATOM H37  HE1      0.15  ! _/

BOND N1  C18 N1  C19                                 !
DOUBLE N1 C15                                        !
BOND C19 H13 C19 H14 C19 H15 C18 H10 C18 H11 C18 H12 !
BOND C15 C16 C16 C17 C17 C12 C12 C13 C13 C14 C14 C15 ! RING 1 CARBONS
BOND C14 H7  C16 H8  C17 H9                          ! RING 1 Hs
BOND C12 C11 C11 C8  C8  C9  C9  C10 C10 C13         ! RING 2 CARBONS
BOND C11 H6  C10 C33 C10 C34                         ! RING 2 SUBS
BOND C34 H44 C34 H45 C34 H46 C33 H41 C33 H42 C33 H43 !   "  "  "
BOND C8  C1  C1  C4  C4  C3  C3  C2  C2  C9          ! RING 3 CARBONS
BOND C1  H1  C2  H2                                  ! RING 3 Hs
BOND C4  C5  C5  C6  C6  C7  C7  N2  N2  C3          ! RING 4 CARBONS/N
BOND C5  H3  C5  H40 C6  H4  C6  H38 C7  H39 C7 H5   ! RING 4 Hs
BOND N2  C20 C20 H16 C20 H17 C20 C21                 !
BOND C21 H18 C21 H19 C21 C22 C22 H20 C22 H21         !
BOND C22 C23 C23 N3  N3  H22 N3  C24                 !
DOUBLE   C23 O1                                      !
BOND C24 H23 C24 H24 C24 C25 C25 H25 C25 H26 C25 C26 !
BOND C26 H27 C26 H28 C26 C27 C27 H29 C27 H30 C27 C28 !
BOND C28 H31 C28 H32 C28 C29 C29 H33 C29 H34 C29 N4  !
BOND N4  H35 N4  C30 C30 C31 C31 H36 C32 H37         !
DOUBLE   C30 O2  C31 C32                             !

PRES LNK1         -0.00  ! patch for linking ATTO 610 to THY C5M
                                               !
GROUP                         !
ATOM 2N1   NN2B   -0.34  !
ATOM 2C6   CN3     0.17  !
ATOM 2H6   HN3     0.17  !
ATOM 2C2   CN1T    0.51  !
ATOM 2O2   ON1    -0.41  !
ATOM 2N3   NN2U   -0.46  !
ATOM 2H3   HN2     0.36  !
ATOM 2C4   CN1     0.50  !
ATOM 2O4   ON1    -0.45  !
ATOM 2C5   CN3T   -0.15  !
ATOM 2C5M  CN9    -0.04  !
ATOM 2H52  HN9     0.07  !
ATOM 2H53  HN9     0.07  !
BOND 1C32  2C5M
DELETE ATOM 2H51
```
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!The End!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!