Electronic Supplementary Information for:

Near-infrared fluorescent probe reveals decreased mitochondrial polarity during mitophagy

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1 Apparatus and reagents

NMR spectra were conducted on Bruker Fourier 300, Bruker Avance III HD 400 or 600 spectrometer in MeOD-d$_4$ or DMSO-d$_6$ (Cambridge Isotope Laboratories). High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on an APEX IV FTMS instrument (Bruker, Daltonics). UV-vis absorption spectra were recorded on a UV-2600 spectrophotometer (Shimadzu, Japan) in 1-cm quartz cells. Fluorescence spectra were acquired on a Hitachi F-4600 spectrophotometer in 1 × 1 cm quartz cells with both excitation and emission slit widths of 10 nm and a PMT voltage of 400 V. MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] analyses were conducted on a microplate reader (BIO-TEK Senergy HT, U.S.A.). Confocal fluorescence images were performed on an FV 1200-IX83 confocal laser scanning microscope (Olympus, Japan) and image processing was carried out with Olympus software (FV10-ASW).

Unless otherwise specified, all reagents, including metal ions, thiols, H$_2$O$_2$, and other chemicals, were purchased from J&K Scientific Ltd., Beijing Chemical Plant or Sigma-Aldrich and used as received. Rhodamine 123 (Rh 123) and ER-Tracker Green (ER Green) were ordered from Thermo Fisher. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) and rapamycin was obtained from MedChem Express. MitoTracker Green FM (Mito Green), LysoTracker Green DND-26 (DND-26), human cervical cancer cells (HeLa), human fetal lung fibroblast 1 (HFL-1), human liver carcinoma cells (HepG2), human normal liver cells (L-O2), Dulbecco’s modified Eagle’s media (DMEM), Ham's F-12K (Kaighn's) medium, trypsin/EDTA solution, were purchased from KeyGEN BioTECH Co., Ltd, Nanjing, China. The preparation of reactive oxygen species and their concentration determinations were following the reported method (Li et al, Anal. Chem. 2017, 89, 5519). Ultrapure water (over 18 MΩ·cm) produced by a Milli-Q reference system (Millipore) was used throughout the whole experiments.

2 Synthesis of fluorescent probe HXPI-P and HXPI-M

As depicted in Scheme S1, HXPI-P, HXPI-M and HXPI-E were synthesized in a single step.
Scheme S1. Synthetic procedure of HXPI-P, HXPI-M and HXPI-E.

Synthesis of HXPI-P. 2,4-Dihydroxybenzoic acid (69 mg, 0.45 mmol) and IR-780 (150 mg, 0.23 mmol) were dissolved in dry DMF (5.0 mL) and stirred at room temperature. Triethylamine (0.20 mL) was added slowly and the reaction mixture was warmed up to 110 °C under argon protection. After 1 h, the solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography (CH₂Cl₂/CH₃OH, 20:1), affording HXPI-P (31 mg, 30%) as blue solid. ¹H NMR of HXPI-P (400 MHz, DMSO-d₆; Fig. S1) δ 8.53 (d, J = 16.0 Hz, 1H), 7.97 (s, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.0 Hz, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 6.67 (s, 1H), 6.39 (d, J = 12.0 Hz, 1H), 4.28 (t, J = 8.0 Hz, 2H), 2.70-2.65 (m, 4H), 1.83-1.78 (m, 4H), 1.74 (s, 6H), 0.98 (t, J = 8.0 Hz, 3H). ¹³C NMR of HXPI-P (150 MHz, CD₃OD; Fig. S2) δ 176.1, 171.0, 162.2, 164.9, 159.9, 154.6, 144.0, 140.5, 140.2, 133.0, 128.9, 127.2, 125.3, 124.3, 120.9, 116.2, 112.8, 112.1, 110.9, 101.7, 100.4, 49.0, 44.6, 27.0, 25.4, 22.1, 19.3, 18.8, 8.67. HR-ESI-MS: m/z calcd for [C₂₉H₂₉N₉NaO₄]⁺, 478.1994; found, 478.1989 (Fig. S3).

Fig. S1 ¹H NMR spectrum of HXPI-P (400 MHz, DMSO-d₆, 298 K)
**Fig. S2** $^{13}$C NMR spectrum of HXPI-P (150 MHz, CD$_3$OD, 298 K).

**Fig. S3** HR-ESI-MS of HXPI-P

**Synthesis of HXPI-M.** HXPI-M was obtained by the condensation of 3,5-dihydroxybenzoic acid and IR-780 with same procedure as mentioned above. Yield: 50%. $^1$H NMR of HXPI-M (400 MHz, CD$_3$OD; Fig. S4) $\delta$ 8.72 (d, $J = 13.2$ Hz, 1H), 8.31 (s, 1H), 7.67 (m, 1H), 7.57 (m, 2H), 7.46-7.43 (m, 2H), 6.97 (s, 1H), 6.52 (d, $J = 13.6$ Hz, 1H), 4.35 (m, 2H), 2.78-2.71 (m, 4H), 1.95 (m, 4H), 1.83 (s, 6H), 1.09 (m, 3H). $^{13}$C NMR of HXPI-M (150 MHz, CD$_3$OD; Fig. S5) $\delta$ 178.1, 167.2, 160.7, 160.4,
159.9, 155.0, 145.1, 142.1, 141.6, 131.7, 128.9, 127.2, 122.4, 116.61, 116.59, 114.4, 114.1, 112.7, 105.3, 103.8, 50.7, 46.3, 29.1, 26.8, 23.6, 20.9, 20.3, 10.2. HR-ESI-MS: m/z calcd for [C_{29}H_{30}NO_4]^+; 456.2169; found, 456.2169 (Fig. S6).

**Fig. S4** $^1$H NMR spectrum of HXPI-M (400 MHz, CD$_3$OD, 298 K)

**Fig. S5** $^{13}$C NMR spectrum of HXPI-M (150 MHz, CD$_3$OD, 298 K).
Synthesis of HXPI-E. HXPI-E was obtained by the condensation of methyl 2,4-dihydroxybenzoate and IR-780 with the same procedure as mentioned above. Yield: 70%. $^1$H NMR of HXPI-E (300 MHz, CD$_3$OD; Fig. S7) δ 8.69 (m, 1H), 7.97 (m, 1H), 7.72 (m, 2H), 7.56 (m, 2H), 7.22 (d, J = 3.3 Hz, 1H), 6.89 (m, 1H), 6.69 (d, J = 15.3 Hz, 1H), 4.47 (t, J = 7.5 Hz, 2H), 4.00 (s, 3H), 2.74 (m, 4H), 2.04-1.86 (m, 4H), 1.84 (s, 6H), 1.11 (t, 3H). $^{13}$C NMR of HXPI-E (75 MHz, CD$_3$OD; Fig. S8) δ 180.6, 170.7, 165.2, 160.5, 158.4, 147.5, 143.9, 142.8, 132.2, 131.1, 130.4, 129.2, 129.1, 124.0, 116.4, 116.3, 114.8, 111.7, 107.3, 104.5, 53.36, 52.59, 30.08, 28.14, 25.06, 21.50, 11.61, 9.29. HR-ESI-MS: m/z calcd for [C$_{30}$H$_{32}$NO$_4$]$^+$, 470.2326; found, 470.2323 (Fig. S9).
Determination of octanol-water partition coefficient

The 1-octanol-water partition coefficient (Log $P_{\text{oct}}$) was calculated according to a reported procedure (Jung et al, *J. Am. Chem. Soc.* 2017, 139, 7595). The log $P_{\text{oct}}$ values for HXPI-P were found to be 0.76 as noted in the main text.

General procedure for polarity measurement

Stock solution (1.0 mM) of HXPI-P was prepared in DMSO. For spectroscopic measurement, 30 μL stock solution of the probe was well mixed with 3.0 mL common solvents respectively, and then
the mixture was transferred to a 1-cm quartz cell to measure absorbance against the corresponding reagent blank or fluorescence spectra with $\lambda_{ex} = 635$ nm.

5 Establishment of the polarity calibration curve

A series of solutions with different polarity were prepared by mixing 1,4-dioxane and water at varied volume ratios. 30 $\mu$L stock solution of the probe was added into 3.0 mL of the mixed solutions and fluorescence spectra were collected with $\lambda_{ex} = 635$ nm. A calibration curve was plotted by the emission maximum shifts or the fluorescence intensity ratios at two wavelengths against the dielectric constant of the mixed system.

6 Computational method

All the theoretical calculations were carried out with the density-functional theory (DFT) and time-dependent density-functional theory (TD-DFT) methods in Gaussian 09 package. Both the geometry optimization of ground state and first excited state were performed at the B3LYP method (Lee et al, Phys. Rev. B. 1988, 37, 785) with 6-31+G (d) basis set (McLean et al, J. Chem. Phys. 1980, 72, 5639) in the PCM solvent continuum models (Water and Dioxane; Cancès et al, J. Chem. Phys. 1997, 107, 3032). The vibration frequency calculations were carried out at the same computational method and basis set to make sure that the optimized structures were true energy minima. Based on the final optimized structures of ground state and first excited state, the dipole moment were also calculated at the same computational method and basis set.

7 Culture of Cells

HeLa, HepG2 and L-O2 cells were cultured using DMEM media. HFL-1 cells were propagated in Ham’s F12k media. The media were all supplemented with 10% (v/v) fetal bovine serum (FBS, GIBCO) and 1% (v/v) penicillin-streptomycin. Cells were grown in a humidified 5% CO$_2$ incubator at 37 °C.

8 Cytotoxicity Assay.

The cytotoxicity of HXPI-P to HeLa cells was examined by standard MTT assay according to the previous report (Wan et al, Angew. Chem. In. Ed. 2014, 53, 10916).

9 Intracellular Fluorescence Imaging.

Cells were seeded in 15 mm glass-bottom culture dishes for 24 h to adhere before experiments. Before use, the cells were washed with FBS-free media for three times. For imaging, the cells were incubated with 5.0 $\mu$M HXPI-P in incubator for 10 min and then the cells were subjected to
fluorescence imaging experiments using a 100× or 60× oil immersion objective lens. The pixel intensity in each fluorescence image was measured and averaged from at least five cells.

10 Co-localization Experiments

Cells (HeLa and HFL-1) seeded in glass-bottom culture dishes were simultaneously incubated with HXPI-P (2.0 μM) and Mito Green (500 nM)/ DND-26 (1.0 μM)/ ER Green (1.0 μM) for 10 min in media without FBS. The cells images were obtained with excitations at either 635 nm (for HXPI-P) or 488 nm (for Mito Green, DND-26 and ER Green); the corresponding fluorescence emissions were collected at 650-750 nm (for HXPI-P) and 500-550 nm (for Mito Green, DND-26 and ER Green), respectively.

11 Supplementary Figures

Table S1. Photo-physical properties of HXPI-P in various solvents at 25 °C.

| HXPI-P (Solvent) | λ\text{abs}^{a} /nm | ε\text{max}(M^{-1}cm^{-1}) | λ\text{em}^{b} /nm | φ^{c} | stocks | Shift/nm |
|------------------|----------------------|----------------------------|---------------------|------|--------|----------|
| Water            | 648                  | 17100                      | 672                 | 0.02 |        | 24       |
| ethylene glycol  | 662                  | 59900                      | 694                 | 0.19 |        | 32       |
| MeOH             | 662                  | 67200                      | 710                 | 0.14 |        | 48       |
| EtOH             | 670                  | 76600                      | 715                 | 0.29 |        | 45       |
| 1-butanol        | 676                  | 94800                      | 717                 | 0.45 |        | 41       |
| DCM              | 702                  | 118700                     | 730                 | 0.98 |        | 28       |
| diethyl ether    | 714                  | 88700                      | 735                 | 0.49 |        | 21       |
| 1,4-dioxane      | 708                  | 94500                      | 733                 | 0.73 |        | 25       |

\(^a\)The maximal absorption of the dye. \(^b\)The maximal emission of the dyes. \(^c\)φ is the relative fluorescence quantum yield estimated by using indocyanine green (ICG, φ = 0.13 in DMSO) as a fluorescence standard (Reindl et al, J. Photochem. Photobiol. A: Chem. 1997, 105, 65).

**Fig. S10** Color changes of HXPI-P (10 μM) in the mixture of water and 1,4-dioxane. The percentage indicates the volume fraction of water.
\[ h c \tilde{\nu}_{max} = -\frac{2\mu_e(\mu_e-\mu_g)}{\alpha^3} \Delta f + \text{constant} \]  
(Equation S1)

Equation S1 is the Lippert-Metaga equation, which neglects the mean solute polarizability in the excited and ground states (Singh et al, Photochem. Photobiol. 1998, 68, 32). Here, \( h \) is the Planck constant, \( c \) is the light speed in vacuum, \( \tilde{\nu}_{max} \) is the solvent-equilibrated fluorescence maxima (wave number), \( \mu_e \) and \( \mu_g \) are the dipole moments of excited and ground states, \( \alpha \) is the Onsager cavity radius, \( \Delta f \) is the orientational polarizability of solvents, and 
\[ \Delta f = \frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{4n^2+2} \]  
(\( \varepsilon \) is the solvent dielectric constant, and \( n \) is the solvent refractive index). Generally speaking, \( \Delta f \) is positively correlated to the dielectric constant \( \varepsilon \). As can be seen from Fig. 1C and Fig. 2C, \( \tilde{\nu}_{max} \) will increase in solvents with higher polarity. Therefore, the slope of Equation S1 is positive. That is to say, there is a decrease in the dipole moment upon excitation. In such cases, the ground state will be energetically stabilized with respect to the excited state, and hence, a significant blue shift of the fluorescence will be observed in high polarity solvents.

**Table S2.** Theoretical calculation for the dipole moments of HXPI-P in water and 1,4-dioxane from B3LYP functional with 6-31+G (d) basis.

| Solvent | State                  | X    | Y    | Z    | \( \mu \) (D) |
|---------|------------------------|------|------|------|--------------|
| Water   | Ground State           | 6.4024 | 4.9759 | 0.8184 | 8.1498       |
| Water   | First Excited State    | 3.3411 | 5.6043 | 1.1249 | 6.6209       |
| Dioxane | Ground State           | 5.2634 | 4.0294 | 0.8032 | 6.6771       |
| Dioxane | First Excited State    | 2.5029 | 4.5243 | 0.9423 | 5.2556       |

**Fig. S11** Fluorescence response of HXPI-P (10 \( \mu \)M) to pH, viscosity and various biological coexisting substances. (A) Plot of \( I_{730}/I_{670} \) vs pH in the range of pH 2.0 to pH 9.2. (B) Plot of \( I_{730}/I_{670} \) vs viscosity.
in the range of $\eta = 22.1$ (glycol) to $\eta = 1495$ (glycerol). (C) Fluorescence response to various substances: water, KCl (150 mM), CaCl$_2$ (2.0 mM), MgCl$_2$ (2.0 mM), CuCl$_2$ (100 $\mu$M), ZnCl$_2$ (100 $\mu$M), glucose (10 mM), glutathione (1.0 mM), cysteine (100 $\mu$M), vitamin C (1.0 mM), ONOO$^-$ (100 $\mu$M), O$_2$ (100 $\mu$M), H$_2$O$_2$ (100 $\mu$M), Na$_2$S (100 $\mu$M), NaHS (100 $\mu$M), Na$_2$SO$_3$ (100 $\mu$M), NaHSO$_3$ (100 $\mu$M), KCN (100 $\mu$M) and 1,4-dioxane. $\lambda_{ex} = 635$ nm. Data are expressed as the mean ± SD of three separate measurements.

**Fig. S12** Structures of (A) probe HXPI-P, (B) control compound HXPI-M and (C) control compound HXPI-E.

**Fig. S13** Fluorescence responses of HXPI-M and HXPI-E (10 $\mu$M) to pH. (A) Fluorescence emission spectra of HXPI-M in phosphate buffer (20 mM) at different pH values. (B) Plot of $\lambda_{em}$ vs pH of HXPI-
M in the range of pH 2.0 to pH 9.2. (C) Fluorescence emission spectra of HXPI-E in phosphate buffer (20 mM) at different pH values. (D) Plot of $\lambda_{em}$ vs pH of HXPI-E in the range of pH 2.4 to pH 8.9. The percentage indicates the volume fraction. $\lambda_{ex}$ = 635 nm. Data are expressed as the mean ± SD of three separate measurements.

**Fig. S14** Cell viability of HeLa cells treated with HXPI-P at varied concentrations for 24 h. The results are the mean ± SD of five separate measurements.

**Fig. S15** Photostability comparison of HXPI-P and Mito Green in HeLa cells. (A) Images of HeLa cells stained with HXPI-P (5.0 μM, $\lambda_{ex}$ = 635 nm, $\lambda_{em}$ = 650-750 nm). (B) Changes of fluorescence intensity of HXPI-P in cells. (C) Images of HeLa cells stained with Mito Green (500 nM, $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-550 nm). (D) Changes of fluorescence intensity of Mito Green in cells. The initial fluorescence intensity (i.e., at about 0 min) is defined as 1.0. The data are expressed as the mean ± SD of three separate measurements. Fluorescence imaging was performed under the continual excitations of 635 or 488 nm for different periods of time (0-30 min). Light power density: 2.0 mW/cm². Scale bars, 20 μm.
Fig. S16 Photostability of HXPI-P (5.0 μM, λ_{ex/em} = 635/670 nm) and Mito Green (500 nM, λ_{ex/em} = 488/520 nm) in water under continuous irradiation of xenon lamp (150 W).

Fig. S17 Mitochondria-targeting properties of HXPI-P in HFL-1 cells. (A) Colocalization images of HFL-1 cells stained with Mito Green (500 nM, green channel, λ_{ex} = 488 nm, λ_{em} = 500-550 nm) and HXPI-P (2.0 μM, red channel, λ_{ex} = 635 nm, λ_{em} = 650-750 nm), and the correlation of HXPI-P and Mito Green intensities as well as the intensity profiles within the ROI (white line in the merged image of A; Pearson’s coefficient 0.90). (B) Colocalization images of HFL-1 cells stained with DND-26 (1.0 μM, green channel, λ_{ex} = 488 nm, λ_{em} = 500-550 nm) and HXPI-P [as in (A)], and the correlation of HXPI-P and DND-26 intensities as well as the intensity profiles within the ROI (white line in the merged image of B; Pearson’s coefficient 0.54). (C) Colocalization images of HFL-1 cells stained with ER Green (1.0 μM, green channel, λ_{ex} = 488 nm, λ_{em} = 500-550 nm) and HXPI-P [as in (A)], and the correlation of HXPI-P and ER Green intensities as well as the intensity profiles within the ROI (white line in the merged image of C; Pearson’s coefficient 0.80). Scale bar = 20 μm.
**Fig. S18** Mitochondria-targeting properties of HXPI-P in depolarized HFL-1 and HeLa cells. Cells were treated with HXPI-P (2.0 μM) and rhodamine 123 (500 nM) for 10 min (control); the probe and rhodamine 123 treated cells were subjected to CCCP (100 nM) for 5 min to induce mitochondria uncoupling. First row, rhodamine 123 channel (λ<sub>ex</sub> = 488 nm, λ<sub>em</sub> = 500-550 nm); second row, HXPI-P channel (λ<sub>ex</sub> = 635 nm, λ<sub>em</sub> = 650-750 nm). Scale bar = 20 μm.

**Fig. S19** MMP effects on the fluorescence intensity of HXPI-P in HeLa cells. Cells were treated with HXPI-P (5.0 μM) for 10 min (control); the probe-treated cells were then subjected to different concentrations of CCCP for 5 min to induce mitochondria uncoupling. Red channel (λ<sub>em</sub> = 650-750
nm); green channel ($\lambda_{em} = 655-685$ nm); orange channel ($\lambda_{em} = 700-730$ nm); the fourth row shows the ratiometric images between orange and green channels. $\lambda_{ex} = 635$ nm. Scale bar = 20 $\mu$m. (B) Relative pixel intensity of the fluorescence images a-j in panel (A). (C) Relative fluorescence intensity ratios (R) of the corresponding ratio images in panel (A). The pixel intensity and the relative ratio values from the control images are defined as 1.0.

**Fig. S20** Confocal fluorescence images of HFL-1 cells incubated with chloroquine (10 $\mu$M), HXPI-P (5.0 $\mu$M) and MDC (1.0 $\mu$M). (A) MDC ($\lambda_{em} = 450-550$ nm) channel was collected at $\lambda_{ex} = 405$ nm, green ($\lambda_{em} = 655-685$ nm) and red ($\lambda_{em} = 700-730$ nm) channels were collected at $\lambda_{ex} = 635$ nm. The images in the third row are the merged ones of red and green channels. The forth row shows the corresponding ratiometric images between red and green channels. Scale bar = 20 $\mu$m. (B) Polarity changes of mitochondria with time. The fluorescence intensity ratio at 0 min is defined as 1.0. The data are expressed as the mean ± SD of three measurements. Significant differences are performed by Student’s t-test.

**Table S3.** Molecule coordinates of HXPI-P

| Number | Atomic Number | X       | Y       | Z       |
|--------|---------------|---------|---------|---------|
| 1      | 6             | 5.074943| -0.146510| -0.315753|
| 2      | 6             | 6.456033| -0.133154| -0.471213|
| 3      | 6             | 7.145117| -1.355347| -0.485201|
|   |   |   |   |   |
|---|---|---|---|---|
| 4 | 6 | 6.454218 | -2.565288 | -0.343597 |
| 5 | 6 | 5.062767 | -2.589882 | -0.187151 |
| 6 | 6 | 4.402366 | -1.362301 | -0.180471 |
| 7 | 7 | 3.015761 | -1.105323 | -0.049283 |
| 8 | 6 | 2.040422 | -2.187818 | 0.144275 |
| 9 | 6 | 1.807648 | -2.511646 | 1.627906 |
|10 | 6 | 0.838766 | -3.685230 | 1.806848 |
|11 | 6 | 2.749949 | 0.219033 | -0.076757 |
|12 | 6 | 1.437367 | 0.720524 | 0.037782 |
|13 | 6 | 1.117380 | 2.073360 | 0.042726 |
|14 | 6 | 4.071540 | 0.991385 | -0.253742 |
|15 | 6 | 4.383717 | 1.895354 | 0.967926 |
|16 | 6 | 4.095553 | 1.800018 | -1.576044 |
|17 | 6 | -2.550975 | 4.360284 | 0.561854 |
|18 | 6 | -1.341850 | 4.973055 | -0.149486 |
|19 | 6 | -0.061939 | 4.261934 | 0.294404 |
|20 | 6 | -0.128457 | 2.741648 | 0.150085 |
|21 | 6 | -1.391009 | 2.145257 | 0.142762 |
|22 | 6 | -2.622757 | 2.871383 | 0.315274 |
|23 | 6 | -3.809862 | 2.196226 | 0.258770 |
|24 | 6 | -3.852321 | 0.781491 | 0.037598 |
|25 | 6 | -2.625738 | 0.107526 | -0.122666 |
|26 | 8 | -1.447339 | 0.790728 | -0.057642 |
|27 | 6 | -2.562646 | -1.259253 | -0.354703 |
|28 | 6 | -3.745490 | -2.002889 | -0.425818 |
|29 | 8 | -3.704025 | -3.336000 | -0.655808 |
|30 | 6 | -5.007818 | -1.360961 | -0.262732 |
|31 | 6 | -5.025932 | 0.019774 | -0.037333 |
|32 | 6 | -6.286316 | -2.112167 | -0.324608 |
|33 | 8 | -6.414084 | -3.316376 | -0.473083 |
|34 | 8 | -7.366670 | -1.300033 | -0.191797 |
|35 | 1 | 6.999983 | 0.801192 | -0.580010 |
|36 | 1 | 8.224600 | -1.362388 | -0.605145 |
|37 | 1 | 7.001574 | -3.503432 | -0.353019 |
|38 | 1 | 4.539671 | -3.533142 | -0.073570 |
|39 | 1 | 2.770664 | -2.748612 | 2.096481 |
|40 | 1 | 1.220388 | -4.593375 | 1.324590 |
|41 | 1 | -0.145422 | -3.462433 | 1.377846 |
|42 | 1 | 0.696780 | -3.905118 | 2.870510 |
|43 | 1 | 1.418427 | -1.619949 | 2.134244 |
|44 | 1 | 2.422759 | -3.062667 | -0.387423 |
|45 | 1 | 1.111224 | -1.905214 | -0.354099 |
|46 | 1 | 0.639527 | 0.000634 | 0.142398 |
First Excited State, Water:

| Number | Atomic Number | X       | Y       | Z       |
|--------|---------------|---------|---------|---------|
| 1      | 6             | 5.039673| -0.194715| -0.364484|
| 2      | 6             | 6.409542| -0.229966| -0.576341|
| 3      | 6             | 7.066441| -1.471705| -0.565544|
| 4      | 6             | 6.348952| -2.659073| -0.340385|
| 5      | 6             | 4.970902| -2.642212| -0.125618|
| 6      | 6             | 4.330384| -1.392675| -0.145626|
| 7      | 7             | 2.983459| -1.104312| 0.025928 |
| 8      | 6             | 1.984046| -2.136277| 0.311584 |
| 9      | 6             | 1.785167| -2.376783| 1.818405 |
| 10     | 6             | 0.758225| -3.483581| 2.079687 |
| 11     | 6             | 2.742692| 0.257786 | -0.054420|
| 12     | 6             | 1.464358| 0.787218 | 0.076713 |
| 13     | 6             | 1.135516| 2.165961 | 0.035810 |
| 14     | 6             | 4.076404| 0.976158 | -0.310405|
| 15     | 6             | 4.468046| 1.921040 | 0.858874 |
| 16     | 6             | 4.077895| 1.741828 | -1.660005|
| 17     | 6             | -2.584822| 4.351960 | 0.584498 |
| 18     | 6             | -1.390553| 4.998163 | -0.121759|
| 19     | 6             | -0.095285| 4.306295 | 0.308874 |
| 20     | 6             | -0.112414| 2.794116 | 0.136306 |
| 21     | 6             | -1.402518| 2.178367 | 0.124643 |
|   |   |       |        |        |
|---|---|-------|-------|-------|
| 22 | 6 | -2.614004 | 2.862939 | 0.335349 |
| 23 | 6 | -3.817503 | 2.159422 | 0.300710 |
| 24 | 6 | -3.831776 | 0.765754 | 0.046918 |
| 25 | 6 | -2.585821 | 0.119571 | -0.185434 |
| 26 | 8 | -1.427184 | 0.821602 | -0.138480 |
| 27 | 6 | -2.506370 | -1.233828 | -0.473830 |
| 28 | 6 | -3.675815 | -2.003774 | -0.530513 |
| 29 | 8 | -3.612923 | -3.321438 | -0.804820 |
| 30 | 6 | -4.949327 | -1.398811 | -0.297583 |
| 31 | 6 | -4.993299 | -0.030677 | -0.016998 |
| 32 | 6 | -6.209433 | -2.181166 | -0.349652 |
| 33 | 8 | -6.315498 | -3.371110 | -0.599633 |
| 34 | 8 | -7.298750 | -1.416321 | -0.081007 |
| 35 | 1 | 6.973822 | 0.682463 | -0.748394 |
| 36 | 1 | 8.138999 | -1.514855 | -0.730610 |
| 37 | 1 | 6.874580 | -3.609584 | -0.331491 |
| 38 | 1 | 4.430511 | -3.565437 | 0.052202 |
| 39 | 1 | 2.749458 | -2.646132 | 2.266512 |
| 40 | 1 | 1.075756 | -4.434540 | 1.634506 |
| 41 | 1 | -0.222483 | -3.225340 | 1.662289 |
| 42 | 1 | 0.632399 | -3.643172 | 3.156206 |
| 43 | 1 | 1.462649 | -1.441666 | 2.292011 |
| 44 | 1 | 2.311996 | -3.054604 | -0.182322 |
| 45 | 1 | 1.044674 | -1.846728 | -0.165151 |
| 46 | 1 | 0.655336 | 0.090050 | 0.242406 |
| 47 | 1 | 1.961633 | 2.864055 | -0.036243 |
| 48 | 1 | 4.454446 | 1.388347 | 1.814969 |
| 49 | 1 | 3.794853 | 2.778747 | 0.932221 |
| 50 | 1 | 5.481045 | 2.301784 | 0.691749 |
| 51 | 1 | 3.815524 | 1.075799 | -2.488131 |
| 52 | 1 | 5.077761 | 2.147407 | -1.847564 |
| 53 | 1 | 3.370666 | 2.575210 | -1.650616 |
| 54 | 1 | -2.514746 | 4.535484 | 1.667344 |
| 55 | 1 | -3.531481 | 4.791899 | 0.252737 |
| 56 | 1 | -1.339035 | 6.064814 | 0.121494 |
| 57 | 1 | -1.516025 | 4.916652 | -1.209621 |
| 58 | 1 | 0.761424 | 4.715887 | -0.238017 |
| 59 | 1 | 0.089554 | 4.528558 | 1.372555 |
| 60 | 1 | -4.755653 | 2.684433 | 0.457236 |
| 61 | 1 | -1.539885 | -1.690537 | -0.660221 |
| 62 | 1 | -2.687847 | -3.594273 | -0.937228 |
| 63 | 1 | -5.952856 | 0.441324 | 0.156416 |
| 64 | 1 | -8.082265 | -1.995734 | -0.135896 |
Ground State, Dioxane:

| Number | Atomic Number | X       | Y       | Z        |
|--------|---------------|---------|---------|----------|
| 1      | 6             | 5.045452| -0.168017| -0.396297|
| 2      | 6             | 6.415656| -0.178268| -0.627710|
| 3      | 6             | 7.091877| -1.406957| -0.621777|
| 4      | 6             | 6.400753| -2.600722| -0.383150|
| 5      | 6             | 5.020710| -2.602219| -0.148270|
| 6      | 6             | 4.372217| -1.368823| -0.165382|
| 7      | 7             | 2.995863| -1.089977| 0.027973 |
| 8      | 6             | 2.022920| -2.147104| 0.330210 |
| 9      | 6             | 1.872446| -2.408387| 1.836571 |
| 10     | 6             | 0.863774| -3.527719| 2.115396 |
| 11     | 6             | 2.739101| 0.235869 | -0.052991|
| 12     | 6             | 1.437270| 0.751748 | 0.088855 |
| 13     | 6             | 1.112195| 2.104768 | 0.062646 |
| 14     | 6             | 4.057040| 0.983479 | -0.330544|
| 15     | 6             | 4.439557| 1.931407 | 0.837049 |
| 16     | 6             | 4.016284| 1.741723 | -1.681230|
| 17     | 6             | -2.578420| 4.346498| 0.606763 |
| 18     | 6             | -1.378679| 4.980755| -0.102445|
| 19     | 6             | -0.088498| 4.281503| 0.331434 |
| 20     | 6             | -0.136496| 2.761406| 0.176701 |
| 21     | 6             | -1.394932| 2.150413| 0.162044 |
| 22     | 6             | -2.632788| 2.858856| 0.345277 |
| 23     | 6             | -3.812228| 2.166931| 0.284598 |
| 24     | 6             | -3.840122| 0.757844| 0.038834 |
| 25     | 6             | -2.607138| 0.102651| -0.143313|
| 26     | 8             | -1.435869| 0.799196| -0.061546|
| 27     | 6             | -2.529749| -1.256649| -0.414582|
| 28     | 6             | -3.706215| -2.011677| -0.500256|
| 29     | 8             | -3.660345| -3.330000| -0.773789|
| 30     | 6             | -4.973770| -1.388078| -0.307730|
| 31     | 6             | -5.007413| -0.017525| -0.048417|
| 32     | 6             | -6.244193| -2.162953| -0.378576|
| 33     | 8             | -6.345361| -3.364553| -0.519503|
| 34     | 8             | -7.334604| -1.358856| -0.259180|
| 35     | 1             | 6.961902| 0.743107 | -0.810700|
| 36     | 1             | 8.162398| -1.432026| -0.801951|
| 37     | 1             | 6.939612| -3.543495| -0.377738|
| 38     | 1             | 4.497654| -3.533454| 0.041906 |
| 39     | 1             | 2.851458| -2.672269| 2.254543 |
### First Excited State, Dioxane:

| Number | Atomic Number | X       | Y       | Z       |
|--------|---------------|---------|---------|---------|
| 1      | 6             | 5.031732| -0.221367| -0.384194|
| 2      | 6             | 6.396304| -0.264214| -0.622389|
| 3      | 6             | 7.049793| -1.507170| -0.610027|
| 4      | 6             | 6.333617| -2.689937| -0.355032|
| 5      | 6             | 4.961360| -2.665699| -0.112499|
| 6      | 6             | 4.322460| -1.415162| -0.136094|
| 7      | 7             | 2.979705| -1.120552| 0.057311 |
| 8      | 6             | 1.975362| -2.133003| 0.377409 |
| 9      | 6             | 1.734780| -2.295313| 1.889320 |
| 10     | 6             | 0.648567| -3.336220| 2.179422 |
| 11     | 6             | 2.741108| 0.248509 | -0.039780|
| 12     | 6             | 1.472690| 0.780004 | 0.098389 |
| 13     | 6             | 1.143322| 2.167470 | 0.052583 |
| 14     | 6             | 4.076429| 0.956472 | -0.323681|
|   |   | 4.494261 | 1.904849 | 0.833504 |
|---|---|----------|----------|----------|
|15 | 6  | 4.060337 | 1.713678 | -1.677311|
|16 | 6  | -2.576008 | 4.354955 | 0.569345 |
|17 | 6  | -1.378337 | 4.997493 | -0.135703|
|18 | 6  | -0.084444 | 4.311988 | 0.310067 |
|19 | 6  | -0.094977 | 2.797862 | 0.140261 |
|20 | 6  | -1.391370 | 2.179552 | 0.125292 |
|21 | 6  | -2.601234 | 2.862920 | 0.331995 |
|22 | 6  | -3.805016 | 2.155250 | 0.296269 |
|23 | 6  | -3.818022 | 0.763430 | 0.042043 |
|24 | 6  | -2.572598 | 0.120557 | -0.193561|
|25 | 8  | -1.412386 | 0.826085 | -0.141437|
|26 | 6  | -2.491438 | -1.230566| -0.491504|
|27 | 6  | -3.660986 | -2.002136| -0.554712 |
|28 | 8  | -3.603961 | -3.313833| -0.844724 |
|29 | 6  | -4.933598 | -1.399377| -0.311490 |
|30 | 6  | -4.980245 | -0.036781| -0.024036 |
|31 | 6  | -6.193050 | -2.194565| -0.360948 |
|32 | 8  | -6.281637 | -3.384720| -0.585970 |
|33 | 8  | -7.285982 | -1.424439| -0.115736 |
|34 | 1  | 6.960376  | 0.643881 | -0.816788 |
|35 | 1  | 8.118289  | -1.556031| -0.796965 |
|36 | 1  | 6.857518  | -3.641137| -0.345315 |
|37 | 1  | 4.423829  | -3.586211| 0.088059  |
|38 | 1  | 2.676426  | -2.589416| 2.368608  |
|39 | 1  | 0.915074  | -4.319713| 1.773245  |
|40 | 1  | -0.315020 | -3.040362| 1.745519  |
|41 | 1  | 0.502962  | -3.451572| 3.258514  |
|42 | 1  | 1.452018  | -1.325323| 2.315770  |
|43 | 1  | 2.309787  | -3.078547| -0.058276 |
|44 | 1  | 1.045950  | -1.867359| -0.136495 |
|45 | 1  | 0.660825  | 0.089460 | 0.278381  |
|46 | 1  | 1.975879  | 2.859757 | -0.008659 |
|47 | 1  | 4.490959  | 1.379753 | 1.793978  |
|48 | 1  | 3.830661  | 2.769793 | 0.911992  |
|49 | 1  | 5.508212  | 2.275696 | 0.650894  |
|50 | 1  | 3.785828  | 1.044597 | -2.498992 |
|51 | 1  | 5.057291  | 2.117231 | -1.883484 |
|52 | 1  | 3.354444  | 2.548269 | -1.664239 |
|53 | 1  | 2.516347  | 4.551907 | 1.650466  |
|54 | 1  | -3.520229 | 4.790889 | 0.224905 |
|55 | 1  | -1.330906 | 6.066541 | 0.097199  |
|56 | 1  | -1.497224 | 4.906400 | -1.223490|
|   |   |     |     |     |
|---|---|-----|-----|-----|
| 58| 1 | 0.775291 | 4.724956 | -0.230108 |
| 59| 1 | 0.087208 | 4.540049 | 1.374769  |
| 60| 1 | -4.744338 | 2.679187 | 0.450715  |
| 61| 1 | -1.524228 | -1.684899 | -0.685616 |
| 62| 1 | -2.684349 | -3.588134 | -1.002376 |
| 63| 1 | -5.942337 | 0.429229  | 0.153608  |
| 64| 1 | -8.064842 | -2.010649 | -0.161677 |