Supporting Information: **Canonical DNA minor groove insertion of bisbenzamidine-Ru(II) complexes with chiral selectivity**

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Synthesis

All reagents were acquired from commercial sources: TFA were purchased from Scharlau, CH$_2$Cl$_2$ from Panreac, CH$_3$CN from Merck, Ruthenium (III) Chloride was purchased from Johnson Matthey. The rest of reagents were acquired from Sigma-Aldrich.

Reactions were followed by analytical RP-HPLC with an Agilent 1100 series LC/MS using an Eclipse XDB-C18 analytical column (4.6 x 150 mm, 5 μm). Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD model in positive scan mode. Standard conditions for analytical RP-HPLC consisted on an isocratic regime during the first 5 min, followed by a linear gradient from 5% to 75% of solvent B for 30 min at a flow rate of 1 mL/min (A: water with 0.1% TFA, B: acetonitrile with 0.1% TFA), compounds b4bpy and b5bpy were analyzed in the same equipment but with a linear gradient 5 to 95% of B en 30 min.. Compounds were detected by UV absorption at 220, 270, 304 and 330 nm. Purification were performed by semipreparative RP-HPLC with an Agilent 1100 series LC using a Luna 5u C18(2) 100A (5 μm, 10 x 250 mm) reverse-phase column from Phenomenex. Concentrations were measured using the listed extinction coefficients.

[Ru(bpy)$_2$(py)$_2$]Cl$_2$, and Tetraakis-(2-phenylpyridine-C2,N') (μ-dichloro)diiridium, were synthesized following known protocols Both diastereoisomers were employed. The synthesis of the metal complexes (Δ/Λ-4Ru, Δ/Λ-5Ir and Δ/Λ-4Ir) was adapted from related previously reported methods.

The chiral resolution of [Ru(bpy)$_2$Cl$_2$] was performed by crystallization with disodium (+) or (−) O,O'-dibenzoyltartrate, and the reacted with b4bpy and b5bpy to obtain Δ/Λ-4Ru and Δ/Λ-5Ru optically pure.

Synthesis of b4bpy

2,2’-Bipyridine-4,4’-dicarboxaldehyde (150 mg, 0.71 mmol), 4-aminobenzamidine dihydrochloride (309 mg, 1.5 mmol), and sodium cyanoborohydride (113 mg, 1.8 mmol) were added to a round bottom flask and dissolved in 7 mL of CH₃CN/H₂O 1:1. The reaction mixture was stirred under Ar at rt for 30 min. The solvents were removed under reduced pressure and the resulting residue was purified by preparative reverse-phase chromatography. The combined fractions were concentrated and freeze-dried yielding a pale yellow solid that was identified as the trifluoroacetic salt of the desired product (384 mg, 80%).

¹H NMR (500 MHz, DMSO-d₆ δ): 4.54 (s, 4H), 6.70 (d, J = 8.7 Hz, 4H), 7.41 (d, J = 5.0 Hz, 2H), 7.59 (d, J = 8.9 Hz, 4H), 8.37 (s, 2H), 8.55 (s, 4H), 8.60 (d, J = 5.0 Hz, 2H), 8.77 (s, 4H).

¹³C NMR (DMSO-d₆ δ): 44.7 (CH₂), 111.5 (CH), 113.1 (C), 118.7 (CH), 122.6 (CH), 129.7(CH), 149.1(CH), 150.0 (C), 153.0 (C), 155.0 (C), 158.5 (q, C, TFA), 164.2 (C).

Synthesis of b5bpy

2,2′-Bipyridine-5,5′-dicarboxaldehyde (150 mg, 0.71 mmol), 4-aminobenzamidine dihydrochloride (309 mg, 1.5 mmol), and sodium cyanoborohydride (113 mg, 1.8 mmol) were added to a round bottom flask and dissolved in 11 mL of CH₃CN/H₂O 1:1. The reaction mixture was stirred under Ar at rt for 30 min. The solvents were removed under reduced pressure and the resulting residue was purified by preparative reverse-phase chromatography. The combined fractions were concentrated and freeze-dried yielding a pale yellow solid that was identified as the trifluoroacetic salt of the desired product (336 mg, 70%).

1H NMR (400 MHz, DMSO-d₆ δ): 4.54 (s, 4H), 6.70 (d, J = 8.7 Hz, 4H), 7.41 (d, J = 5.0 Hz, 2H), 7.59 (d, J = 8.9 Hz, 4H), 8.37 (s, 2H), 8.55 (s, 4H), 8.60 (d, J = 5.0 Hz, 2H), 8.77 (s, 4H).

13C NMR (DMSO-d₆ δ): 44.7 (CH₂), 111.5 (CH), 113.1 (C), 118.7 (CH), 122.6 (CH), 129.7(CH), 149.1(CH), 150.0 (C), 153.0 (C), 155.0 (C), 158.5 (q, TFA), 164.2 (C).

Synthesis of Δ- and Λ-4Ru

Δ- or Λ-[Ru(bpy)_2(py)_2] (+) or (−) O,O’-dibenzoyltartrate (34 mg, 0.07 mmol) was placed in a round bottom flask and dissolved in 1.2 mL of H_2O/MeOH 1:1 and Ar was bubbled for 10 min, b4bpy (40 mg, 0.06 mmol) was added and the reaction mixture was stirred under Ar in the absence of light at 80 °C for 12 h. The resulting residue was purified by preparative reverse-phase chromatography. The combined fractions were concentrated and freeze-dried yielding an orange-brown solid that was identified as the trifluoroacetic salt of the desired product (60 mg, 77%).

^1H NMR (400 MHz, D_2O δ): 4, 7.3 (s, 4H), 6.81 (d, J = 8.8 Hz, 4H), 7.46 (q, J = 5.5 Hz, 6H), 7.66 (d, J = 8.7 Hz, 4H), 7.82 (t, J = 6.7 Hz, 4H), 7.88 (d, J = 5.6 Hz, 2H), 8.13 (t, J = 7.3 Hz, 4H), 8.47 (s, 2H), 8.61 (d, J = 8.0 Hz, 4H).

^13C NMR (D_2O δ): 47.816 (CH_2), 115.3 (CH), 117.3 (C), 124.6 (CH), 126.8 (CH), 126.9 (CH), 128.5 (CH), 130.0 (CH), 130.1 (CH), 132.6 (CH), 140.5 (CH), 153.9 (CH), 154.0 (CH), 154.1 (CH), 154.2 (CH), 155.6 (C), 159.7 (C), 159.8 (C), 159.9 (C), 168.1 (C), 187.4 (C).

ESI-HRMS: [M]^{++} calc. for C_{46}H_{42}N_{12}Ru 432.1350 found 432.1347. C_{54}H_{60}F_{12}N_{12}O_{8}Ru (M.W. 1320.0695).
Synthesis of Δ- and Λ-5Ru

Δ- or Λ-[Ru(bpy)₃(py)₂](+) or (–) O,O’-dibenzoyltartrate (34 mg, 0.07 mmol) was placed to a round bottom flask and dissolved in 1.2 mL of H₂O/MeOH 1:1 and Ar was bubbled for 10 min. b5bpy (40 mg, 0.06 mmol) was added and the reaction mixture was stirred under Ar in the absence of light at 80 °C for 12 h. The resulting residue was purified by preparative reverse-phase chromatography. The combined fractions were concentrated and freeze-dried yielding an orange-brown solid that was identified as the trifluoroacetic salt of the desired product (54 mg, 70%).

¹H NMR (400 MHz, D₂O δ): 4.17 (d, J = 18.2 Hz, 2H), 4.35 (d, J = 17.8 Hz, 2H), 6.07 (d, J = 8.5 Hz, 4H), 7.02 (t, J = 6.6 Hz, 2H), 7.09-7.12 (m, 4H), 7.27 (d, J = 8.5 Hz, 4H), 7.36 (d, J = 5.5 Hz, 2H), 7.48 (d, J = 5.5 Hz, 2H), 7.71 (t, J = 7.9 Hz, 4H), 7.81 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.5 Hz, 2H), 7.92 (d, J = 8.5 Hz, 2H), 8.29 (d, J = 8.4 Hz, 2H).

¹³C NMR (D₂O δ): 44.4 (CH₂), 113.2 (CH), 115.3 (C), 125.2 (CH), 125.4 (CH), 125.4 (CH), 128.8 (CH), 128.9 (CH), 130.9 (CH), 138.2 (CH), 139.0 (CH), 139.1 (CH), 141.1(C), 149.7 (CH), 152.3 (CH), 152.5 (CH), 154.2 (C), 157.2 (C), 158.1 (C), 158.2 (C), 166.7 (C).

Synthesis of Δ- and Λ-4Ir

[Ir₂(ppy)₂Cl]₂ (60 mg, 0.056 mmol) was placed to a round bottom flask and dissolved in 1.5 mL of MeOH and Ar was bubbled for 10 min, b4bpy (14 mg, 0.02 mmol) was added and the reaction mixture was stirred under Ar in the absence of light at 80 °C for 12 h. The resulting residue was purified by preparative reverse-phase chromatography. The combined fractions were concentrated and freeze-dried yielding a yellow solid that was identified as the trifluoroacetic salt of the desired product (22 mg, 84%).

$^1$H NMR (400 MHz, CD₃OD δ): 4.67 (s, 4H), 6.27 (dd, $J = 7.5$, 0.5 Hz, 2H), 6.75 (d, $J = 8.9$ Hz, 4H), 6.87 (dt, $J = 7.5$, 1.1 Hz, 2H), 7.01 (dt, $J = 7.7$, 1.0 Hz, 2H), 7.05 (ddd, $J = 7.3$, 6.0, 1.3 Hz, 2H), 7.49 (dd, $J = 5.7$, 1.2 Hz, 2H), 7.62 (d, $J = 8.9$ Hz, 4H), 7.60 (d, $J = 5.3$ Hz, 2H), 7.80 (dd, $J = 7.8$, 0.6 Hz, 2H), 7.85 (dt, $J = 8.4$, 8.0, 1.4 Hz, 2H), 7.93 (d, $J = 5.7$ Hz, 2H), 8.10 (d, $J = 8.2$ Hz, 2H), 8.67 (s, 2H).

$^{13}$C NMR (MeOD-d₄ δ): 46.6 (CH₂), 113.4 (CH), 115.8 (C), 121.0 (CH), 123.6 (CH), 124.2 (CH), 124.4 (CH), 126.0 (CH), 127.6 (CH), 130.9 (CH), 131.5 (CH), 132.7 (CH), 139.6 (CH), 145.2 (C), 149.8 (CH), 151.5 (CH), 151.6 (C), 154.6 (C), 154.8 (C), 157.3 (C), 167.2 (C), 169.4(C).

ESI-HRMS: [M]$^+$ calcd. for C₄₈H₄₂IrN₁₀ = 951.3221 found 951.3211; C₅₄H₄₅F₉IrN₁₀O₆ (M.W. 1293.2011).
Chiral resolution

The chiral isolation of $\Lambda/\Delta$-4Ir was done with a chiral column (Chiralpack®) using a linear gradient of heptanes, ethanol, triethyl amine and trifluoroacetic acid (70:30:0.5:0.3). Each isomer was collected, concentrated under reduced pressure and lyophilized.

**Figure S1.** HPLC trace of racemic mixture $\Lambda/\Delta$-4Ir.

**Figure S2.** HPLC trace of $\Delta$-4Ir.

**Figure S3.** HPLC trace of $\Lambda$-4Ir.
Circular dichroism spectroscopy

Measurements were made with a Jasco-715 Spectropolarimeter coupled with a thermostat Nestlab RTE-111. All the data were recorded at 20 °C Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl. The settings used were: Acquisition range: 600-200 nm, band width: 2.0 nm, resolution: 0.2 nm, accumulation: 2 scans, sensitivity 10 mdeg, response time: 0.25 s, speed: 100 nm/min.

All the CD spectra were measured at 5 µM concentration of the corresponding complex.

![Circular dichroism spectra](image)

**Figure S1.** Circular dichroism spectra of 5 µM solutions of Λ/Δ-4Ru and Λ/Δ-5Ru, and Λ/Δ-4Ir in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl. Λ- isomers in all cases as solid lines; Δ-isomers represented with dashed lines.

Oligonucleotide sequences:

Oligonucleotides were purchased from Thermo Fisher Scientific GmbH on a 0.2 mmol scale as freeze-dried solids. Their concentrations were measured by UV absorption at 260 nm with a BioRad SmartSpec Plus Spectrophotometer. Absorbance was measured twice and concentrations were calculated applying Lambert-Beer’s equation. The molar extinction coefficients of single strand oligonucleotides were calculated by using the following formula,

$$
e_{260} = \{(8.8 \times \#T) + (7.3 \times \#C) + (11.7 \times \#G) + (15.4 \times \#A)\} \times 0.9 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$$

Where #A, #T, #C, #G stand for the number of each type of bases in the DNA strand. Oligonucleotides were hybridized by mixing complementary sequences at equal molar concentration, heating at 90 °C for 10 min and then slowly cooling the mixture to rt over 1h.

Hairpin oligonucleotides were supplied by Thermo Fischer and their sequences were:

- **AAATTT:** 5’-GGC AAATTT CAG TTTTT CTG AAATTT GCC-3’
- **AATTT:** 5’-GGCG AATTT CCG TTTTT GCG AAATTT CGCC-3’
- **AATT:** 5’-GGCG AATT CAGC TTTTT GCTG AATT CGCC-3’
- **ATT:** 5’-GGCGAG ATT CCG TTTTTT GCG AAT CTCGCC-3’
- **GGCCC:** 5’-GGCA GGCC CAGC TTTTT GCTG GGCC TGCC-3’

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DNA-binding studied by circular dichroism spectroscopy

All experiments were made following the same procedure: to 0.5 mL of a 5 μM solution of the select DNA in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, aliquots of a ≈ 500 μM stock solution of the respective compound were successively added, and the circular dichroism spectra was recorded after each addition.

**Figure S2.** CD analysis of DNA interaction of **b4bpy**: Left: CD spectra of a 5 μM solution of **AAATTT** (dashed line), and with 1, 3, and 5 eq. of **b4bpy** (solid lines); Right: CD spectra of a 5 μM solution of **GGCCC** (dashed line) and with 1, 3, and 5 eq. of **b4bpy** (solid lines).

**Figure S3.** CD analysis of DNA interaction of **Δ/Λ-4Ru**: Left: CD spectra of a 5 μM solution of **AAATTT** (dashed line), and with 1, 3, and 5 eq. of **Δ/Λ-4Ru**; Right: CD spectra of a 5 μM solution of **GGCCC** (dashed line) and with 1, 3, and 5 eq. of **Δ/Λ-4Ru** (solid lines).

**Figure S4.** CD analysis of DNA interaction of **Δ/Λ-4Ir**: Left: CD spectra of a 5 μM solution of **AAATTT** (dashed line), and with 1, 3, and 5 eq. of **Δ/Λ-4Ir**; Right: CD spectra of a 5 μM solution of **GGCCC** (dashed line) and with 1, 3, and 5 eq. of **Δ/Λ-4Ir** (solid lines).
**Ultraviolet Spectroscopy**

Measurements were done in a JASCO UV-630 Spectrophotometer coupled with a thermostat PolyScience. All the data were recorded at 20 °C in MilliQ water. The settings used were: Acquisition range: 600-200 nm, band width: 1.5 nm, resolution: 0.2 nm and speed: 400 nm/min.

Ultraviolet spectra of the complexes were measured at 5 μM concentration.

![UV spectra](image)

**Figure S5.** From left to right, UV spectra of racemic 4Ru, racemic 5Ru and racemic 4Ir

**Fluorescence spectroscopy**

**General procedure**

Measurements were made with a Jobin-Yvon Fluoromax-3, (DataMax 2.20) coupled to a Wavelength Electronics LFI–3751 temperature controller, using the following settings: increment: 1.0 nm; integration time: 0.2 s; excitation slit width: 3.0 nm; emission slit width: 6.0 nm; excitation wavelength 329 nm at 20 °C.

In the case of compounds b4bpy and b5bpy the emission spectra were acquired from 345 to 550 nm. In the case of compounds Δ/Λ-4Ru and Δ/Λ-5Ru the emission spectra were acquired from 500 to 750 nm. In the case of compounds Δ/Λ-4Ir the emission spectra were acquired from 450 to 725 nm.

All titrations were made following the same procedure: to 1 mL of a 0.5 μM solution of the select compound in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, aliquots of a ~250 μM stock solution of the respective dsDNA were successively added, and the fluorescence spectra was recorded after each addition. The reported dissociation constants are the average of three experiments.

**Fluorescence titrations of with different DNAs**

Below, we show representative spectra of the titrations of 0.5 μM solutions of all the studied compounds in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl with increasing concentrations of the different oligonucleotides. Next to each titration we include the corresponding titration curves with the best fit to 1:1 binding mode.
Figure S6. Fluorescence emission spectra of 0.5 µM solutions of b4bpy and b5bpy in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl and emission spectra in the presence of increasing concentrations of each of the oligonucleotides; next to each titration we show the plot of the emission intensity at 383 nm with the best-fit curve to a 1:1 binding mode.
Figure S7. Fluorescence emission spectra of 0.5 µM solutions of \textit{\textgamma}-4\textit{Ru} and \textDelta-4\textit{Ru} in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl and emission spectra in the presence of increasing concentrations of each of the oligonucleotides; next to each titration we show the plot of the emission intensity at 605 nm with the best-fit curve to a 1:1 binding mode.
Figure S8. Fluorescence emission spectra of 0.5 µM solutions of Λ-5Ru and Δ-5Ru in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl and emission spectra in the presence of increasing concentrations of each of the oligonucleotides; next to each titration we show the plot of the emission intensity at 605 nm with the best-fit curve to a 1:1 binding mode.
Figure S9. Fluorescence emission spectra of 0.5 µM solutions of Λ-4Ir and Δ-5Ir in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl and emission spectra in the presence of increasing concentrations of each of the oligonucleotides; next to each titration we show the plot of the emission intensity at 550 nm with the best-fit curve to a 1:1 binding mode.
Curve fitting

The typical equation for a 1:1 binding in which an unlabeled ligand (dsDNA) is added over a fluorescent receptor is described by the following equations, if nonspecific binding is ignored:

\[ K_D = \frac{R \times L}{C} \quad (1) \]
\[ C_T = L + C \quad (2) \]
\[ R_T = R + C \quad (3) \]
\[ F_T = F_0 + F_C \times C \quad (4) \]

Where \( R \) is the concentration of the free receptor in the equilibrium; \( R_T \), total receptor concentration (considered constant throughout the titration); \( L \), concentration of the free ligand in the equilibrium; \( L_T \), total concentration of added ligand (DNA); \( K_D \), dissociation constant of the interaction between the receptor and the ligand; \( C \), equilibrium concentration of the ligand-receptor complex; \( F_T \), total observed fluorescence; \( F_0 \), adjustable parameter accounting for the background fluorescence; \( F_C \), adjustable parameter for the labeled ligand-receptor complex molar fluorescence. Solving the system for \( F_T \) and eliminating \( R, L, \) and \( RL \), we obtain the well-known equation 5.

\[ F_T = \frac{1}{2} \left( 2F_0 + F_C \sqrt{K_D^2 + (C_T - R_T)^2 + 2K_D(L_T + R_T)} \right) \quad (5) \]

If non-specific binding of the probe to the DNA is considered, it is better to use an equation in which the total fluorescence, \( F_T \), takes into account this contribution, assuming that the non-specific binding under these concentrations is nonsaturable and linearly dependent on the ligand (DNA) concentration \( (F_L, L_T) \).

\[ F_T = F_0 + F_C \times C + F_L \times L_T \quad (6) \]

The alternative set of equations (1-3, 6) was solved using Mathematica 6.0.1.0 for MacOS X (Wolfram Research), resulting in equation 7, which was used to fit the experimental data using non-linear regression analysis using GraphPad Prism 8.1.1 for Mac OS, GraphPad Software, La Jolla California USA, www.graphpad.com. Plots were also prepared using GraphPad Prism.

\[ F_T = \frac{1}{2} \left( 2F_0 + F_C \times K_D + F_C \times F_T + 2F_L \times L_T + F_C \times R_T - F_C \sqrt{K_D^2 + (C_T - R_T)^2 + 2K_D(L_T + R_T)} \right) \quad (7) \]

Fluorescence displacement titrations

Figure S 10. Fluorescence displacement assay showing the normalized emission spectrum of a mixture of 0.25 µM Hoechst 33258 and 0.5 µM A2T2 (thick continuous line) in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl upon excitation at 329 nm, and a series of spectra in the presence of increasing concentrations of Δ-4Ru. Note the presence of a clear isobestic point at about 577 nm and the progressive increase of the ruthenium luminescence as the Hoechst 33258 emission band at 460 is displaced.

5 M. H. A. Roehrl, J. Y. Wang, G. Wagner, Biochemistry 2004, 43, 16056
Linear Dichroism

LD spectra were measured using a Chirascan spectropolarimeter equipped with an outer rotating Couette cell at 1000 rpm with an optical path length of 1 mm. Baseline spectra, obtained without rotation, were subtracted.

Samples consisted of 10.8 µM solutions of the appropriate enantiomer (Λ-4Ru or Δ-4Ru) and calf thymus DNA (Sigma-Aldrich, 214 µM nucleosides) in 1 mM sodium cacodylate buffer, pH 7, with 10 mM NaCl. The reduced linear dichroism curves (LD' = LD/Aiso) were calculated by dividing the baseline-subtracted LD spectrum with the corresponding (isotropic) absorption spectrum, obtained on a Varian Cary 4000 UV-vis spectrophotometer. The LD spectra were then normalized to perfect orientation by dividing with LD'_{260 nm} (the reduced linear dichroism value at the nucleobase absorption band) and multiplying with $-1.5$ (the theoretical LD' value for a perfectly oriented perpendicularly polarized transition).

Docking studies

Docking calculations were performed with AutoDock 4.2 with the Lamarckian genetic algorithm. Molecular geometries for the ligands and atomic charges were computed with MOPAC16 at the PM6-d3h4 level within a continuum model of water in the singlet ground state.

The most likely conformations for b4bpy, Λ-4Ru and Δ-4Ru were typically extended and were chosen based on the lowest energy conformers found after a simulated annealing procedure with the GFN-xTB (Geometry, Frequency, Non-covalent, eXtended Tight-Binding) program. Results were analyzed and/or rendered with AutoDockTools, Pymol, and UCSF Chimera.

For more details for creating of the DNAs models from fiber data see the 3DNA webpage: [http://x3dna.org/articles/3dna-fiber-models](http://x3dna.org/articles/3dna-fiber-models)

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9 The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC.

DNA Photocleavage setup

The DNA photocleavage was carried out with a high power LEDs. The sample was placed in a standard 10 mm cuvette in a sample holder and was irradiated by one LED unit (M455L3 at 455 nm with 900 mW). The light from the LEDs is collimated by a plano-convex lens with short focal length in order to maximize irradiation power. The setup is straightforward and similar to commercially available equipment. The setup was designed and assembled by Wajih Al-Soufi, from the Department of Physical Chemistry at the University of Santiago de Compostela. A complete description of the apparatus can be found at: [http://www.usc.es/fotofqm/es/node/1134](http://www.usc.es/fotofqm/es/node/1134)

![CAD drawings of the LED device used to irradiate the samples.](image1)

**Figure S11.** CAD drawings of the LED device used to irradiate the samples.

The device contains two High-Power LEDs in our setup, the M365L2 at 365 nm with >190 mW of power, and the M455L3 at 455 nm with 900 mW.

![High-Power LED and spectra.](image2)

**Figure S12.** High-Power LED and spectra. Figures from Thorlabs, Inc.
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**Remarks:**
- The SM1 Coupler (SM1T2) is used to fix the LEDs to the 2" lens tubes.
- The Plano-Convex lenses are mounted with the planar face towards the LED.
- We use uncoated BK7 lenses, with transmission at 365 nm sufficient for illumination.
- The lenses are fixed with retaining rings inside the lens tubes. The distance between lens and LED is critical in order to get a collimated beam. The LA1951 lens has a back focal length of 17.7 mm (Distance between the LED light source and the planar side of the lens.) During the mounting the first retaining ring is screwed into the lens tube down to a depth of approximately 26-27 mm. Then the lens is carefully inserted with the convex side towards the retaining ring. Then the second ring is used to fix the lens. The exact position of the lens inside the tube is not too critical because the lens-LED distance can be slightly varied changing the position of the LED on the SM1 Coupler. The beam diameter has to be adjusted to fill the input window of the sample holder.
- The retaining rings can be rotated in the lens tubes with a screwdriver or much safer with a spanner wrench (Thorlabs SPW602).
- We indicate the /metric/variants of the Thorlabs parts.
- In principle, the plano-convex lenses are optional. Without the lenses the LED could be mounted with shorter lens tubes (SM1L05). However, the lenses increase the irradiance and also isolate the sensitive LED chip from the sample chamber.
- The power supplies can be connected to a timer to control the irradiation time.

**Warnings:**
- The intense UV radiation from the high power LEDs can damage you eyes.
- Make sure that the operation current of the LED Driver does not exceed the maximum allowed value of the LED. Set the LED Current Limit on the driver.