Perylene Diimide as a Precise Graphene-Like Superoxide Dismutase Mimetic—Supporting Information

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Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate.

![Reaction Scheme]

2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate was prepared according to literature procedures. S1 Triethyleneglycol monomethyl ether (4.80 mL, 5.03 g, 30.6 mmol),
triethylamine (6.40 mL, 4.64 g, 45.9 mmol), and DMAP (39 mg, 0.32 mmol) were dissolved in 70 mL of DCM at 0 °C. To this, a solution of tosyl chloride (2.0 M in DCM, 30.0 mL, 11.7 g, 61.2 mmol) was added over a 45 min period via an addition funnel. The reaction was allowed to warm to room temp over 24 h, after which 75 mL of 1 M HCl was added. The solution was extracted with DCM (4 × 30 mL), washed with a saturated sodium bicarbonate solution, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give the crude product as a yellow oil. Purification by silica gel chromatography (100% DCM to remove excess TsCl, followed by 5% MeOH in DCM) gave the pure product as a clear oil (9.11 g, 28.6 mmol, 93% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.80 (d, 2H), 7.37 (d, 2H), 4.18 (t, 2H), 3.70 (t, 2H), 3.60 (m, 6H), 3.54 (t, 2H), 3.38 (s, 3H); 2.46 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 144.76, 132.91, 129.78, 127.84, 71.78, 70.58, 70.40, 69.24, 68.53, 58.86, 21.50.

Synthesis of 2-(2-(2-(methoxyethoxy)ethoxy)ethyl)isoindoline-1,3-dione.

2-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)isoindoline-1,3-dione was prepared according to literature procedures. The tosylate product from the previous step (2.36 g, 7.41 mmol) and potassium phthalimide (2.75 g, 14.8 mmol) were dissolved in 45 mL of DMF and heated at reflux for 24 h, after which 50 mL of water was added and the solution was extracted with DCM (3 × 20 mL). The combined organic layers were washed first with 1 M NaOH then with a saturated NaCl solution, dried over Na₂SO₄, filtered and concentrated to give the phthalimide...
product as a pale yellow oil (2.10 g, 7.15 mmol, 97% yield) that was used in the next step without further purification. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.54 (d, 2H), 7.47 (d, 2H), 3.61 (t, 2H), 3.46 (t, 2H), 3.39-3.20 (m, 6H), 3.18 (t, 2H), 3.04 (s, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 167.61, 161.99, 133.53, 131.63, 122.65, 69.99, 69.94, 67.34, 58.37, 36.81, 35.92, 30.81.

**Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethan-1-amine.**

2-(2-(2-Methoxyethoxy)ethoxy)ethan-1-amine was prepared according to literature procedures.$^{53,54}$ The phthalimide product from the previous step (2.50 g, 8.52 mmol) was dissolved in 10 mL of methanol. To this solution, hydrazine hydrate (1 mL, 20 mmol, 50-60% grade solution) was added and the reaction was heated at reflux for 24 h, after which 100 mL of 1 M NaOH was added and the resulting solution was extracted with DCM (5 x 25 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to give the amine product as a pale yellow oil (1.30 g, 7.96 mmol, 94% yield). $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 3.53 (m, 6H), 3.44 (t, 2H), 3.40 (t, 2H), 3.27 (s, 3H), 2.72 (t, 2H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 72.94, 71.83, 70.48, 70.17, 58.89, 49.70, 41.28.
Synthesis of 350 MW mPEG-4-methylbenzenesulfonate.

Tosylated mPEG was prepared using the same protocol as for 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate. 350 MW average methoxy-PEG (4.00 mL, 4.36 g, 12.5 mmol), triethylamine (5.2 mL, 3.8 g, 37 mmol), and DMAP (76 mg, 0.62 mmol) were dissolved in 30 mL of DCM and cooled to 0°C. To this solution, a solution of tosyl chloride (3.73 M in DCM, 10.0 mL, 7.12 g, 37.3 mmol) was added slowly over 30 min. The reaction was gradually warmed to room temp and stirred for 48 h, after which 50 mL of 1 M HCl was added, and the resulting solution was extracted with DCM (4 x 20 mL), washed with a saturated solution of sodium bicarbonate, dried over Na₂SO₄, and concentrated under reduced pressure to give the crude product as a light brown oil. Purification by silica gel column chromatography (100% DCM to remove excess TsCl, followed by 10% MeOH in DCM) gave the pure product as a clear oil. Characterization data matched that found in literature. ¹H NMR (600 MHz, CDCl₃) δ 7.72 (d, 2H), 7.28 (d, 2H), 4.08 (t, 2H), 3.63-3.47 (m, 28H), 3.30 (s, 3H); 2.38 (s, 3H).
Synthesis of 350 MW mPEG isoindoline-1,3-dione.

![Chemical Structure]

Synthesis of 350 MW mPEG isoindoline-1,3-dione was prepared using the same protocol as for 2-(2-(2-methoxyethoxy)ethoxy)ethylisoindoline-1,3-dione. The tosylated mPEG from the previous step (2.75 g, 5.10 mmol) and potassium phthalimide (2.01 g, 10.8 mmol) were dissolved in 35 mL of DMF and heated to 110°C for 48 h, after which 150 mL of deionized water was added and the resulting solution was extracted with chloroform (3 x 75 mL). The combined organic layers were washed with 2 M NaOH (3x), then with deionized water (5x), then dried over Na₂SO₄, filtered, and concentrated to give the product as a clear oil (1.55 g, 3.07 mmol, 60% yield). Characterization data matched that found in literature.⁶⁷ ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, 2H), 7.64 (d, 2H), 3.81 (t, 2H), 3.65 (t, 2H), 3.55-3.40 (m, 26H), 3.29 (s, 3H).

Synthesis of 350 MW mPEG-1-amine.

![Chemical Structure]

350 MW mPEG-1-amine was prepared using the same procedure as for 2-(2-(2-methoxyethoxy)ethoxy)ethan-1-amine. The phthalimide-mPEG from the previous step (1.60 g, 3.11 mmol) was dissolved in 35 mL of absolute ethanol. To this solution, hydrazine (0.40 mL,
9.3 mmol, 98% grade solution) was added and the reaction was heated to reflux for 24 h, after which 100 mL of 1 M NaOH was added and the resulting solution was extracted with DCM (5 x 50 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give the product as a pale yellow oil (1.17 g, 3.05 mmol, 98% yield). Characterization data matched that found in literature.³⁸ 📑¹H NMR (400 MHz, CDCl₃) δ 3.60-3.54 (m, 26H), 3.47-3.43 (m, 4H), 3.28 (s, 3H), 2.65 (br s, 2H); 📑¹³C NMR (151 MHz, CDCl₃) δ 72.68, 72.53, 72.48, 71.84, 70.47, 70.46, 70.44, 70.41, 70.18, 70.15, 70.04, 61.35, 58.92, 41.45.

Synthesis of N,N'-Di-[10-(3,6,9-trioxadecyl)]perylene-3,4,9,10-bis(dicarboximide).

2-(2-(2-Methoxyethoxy)ethoxy)ethan-1-amine (0.430 g, 2.63 mmol), PTCDA (0.450 g, 1.14 mmol), and zinc acetate (0.280 g, 1.50 mmol) were dissolved in 15 mL of pyridine and heated at reflux for 18 h. After cooling to room temp, the solvent was removed under reduced pressure and the resulting dark purple sticky solid was re-dissolved in ca. 3 mL of DCM. To this was added excess hexanes, which gave a fluffy precipitate that was filtered and rinsed with hexanes and acetone. The resulting solid was dissolved in chloroform, and the remaining
insoluble PTCDA starting material was removed by filtration. The filtrate was concentrated under reduced pressure to give the pure product as a deep purple-pink solid (0.567 g, 0.832 mmol, 73% yield). Characterization data matched that found in the literature. $^\text{59}$ $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.50 (d, 4H), 8.33 (d, 4H), 4.46 (t, 4H), 3.88 (t, 4H), 3.76 (t, 4H), 3.67 (t, 4H), 3.62 (t, 4H), 3.49 (t, 4H), 3.32 (s, 6H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 163.39, 134.42, 129.31, 126.21, 123.30, 123.09, 72.12, 70.89, 70.74, 70.36, 68.13, 59.21, 39.55. ESI-LCMS calc’d for \( \text{C}_{38}\text{H}_{38}\text{N}_2\text{O}_{10} \) 682.25; found 705.01 [\( M + \text{Na}^+ \)].

**Synthesis of N,N’-Di-mPEG-perylene-3,4,9,10-bis(dicarboximide).**

350 MW mPEG-1-amine (0.50 g, 1.3 mmol), PTCDA (220 mg, 0.57 mmol), and zinc acetate (140 mg, 0.75 mmol) were dissolved in 5 mL of pyridine and heated at reflux for 20 h. After cooling to room temp, the solvent was removed under reduced pressure and the resulting solid was re-dissolved in ca. 3 mL of DCM. To this was added excess hexanes, which gave a precipitate that was filtered and rinsed with hexanes. The resulting solid was dissolved in ca. 3 mL of DCM, added dropwise to a large excess of diethyl ether, giving a fluffy purple precipitate,
which was left at 0°C for 12 h before being vacuum filtered to give the pure product as a dark purple solid (511 mg, 0.485 mmol, 85% yield). Characterization data was compared to that for a very similar long-chain-PEG perylene diimide in the literature.\textsuperscript{510} \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.42 (br d, 4H), 8.28 (br d, 4H), 4.43 (t, 4H), 3.86 (t, 4H), 3.73 (t, 4H), 3.68-3.56 (m, 56H), 3.53 (t, 4H), 3.36 (s, 6H). \textsuperscript{13}C NMR (151 MHz, CDCl\textsubscript{3}) \(\delta\) 163.58, 134.80, 129.56, 125.06, 123.42, 123.34, 72.14, 70.83, 70.77, 70.34, 68.11, 59.23, 41.56, 39.55 (multiple and overlapping peaks corresponding to the same type of carbon were observed due to the products being a mixture of various mPEG lengths, most of which were n = 7 or n = 8).

![Figure S1](image)

**Figure S1.** CV in DMSO containing 0.1 M \([(n{-}Bu)4N]\)ClO\textsubscript{4} as a supporting electrolyte at 298 K at a glassy carbon working electrode and platinum wire as quasi reference electrode (Scan rate: 50 mV/s): 10 mM of PEG\textsubscript{8}-PDI in DMSO under N\textsubscript{2}.
Figure S2. UV-vis spectra of PEG$_8$–PDI in DMSO.

Figure S3. CVs in DMSO containing 0.1 M [($n$-Bu)$_4$N]ClO$_4$ as a supporting electrolyte at 298 K at a GC working electrode and platinum wire as a quasi-reference electrode at a scan rate of 100 mV/s. KO$_2$ in DMSO under N$_2$ (red) and after bubbling the solution with N$_2$ for 6 h (blue).
Figure S4. Optical spectra of PEG₈−PDI under H₂O₂ and HClO₄ treatment in DMSO, indicating no reaction.
Figure S5. Optical spectra of PEG₈−PDI⁻⁻ (blue trace) under H₂O₂ treatment in DMSO. Comparison of the final product in gray trace with PEG₈−PDI (black dotted trace) and PEG₈−PDI−OH⁻ (red trace).
Simulation parameters: $g = 2.0019$

1) Ring protons (8): $-1.20G/-0.17G$

2) Sidechain $\alpha$ protons (4): $0.6G/0.08G$

3) Nitrogen atoms (2): $0.04G/-0.056G$

4) Linewidth: 0.23G

**Figure S6.** EPR spectrum of $[\text{PEG}_8\text{--PDI}^-\text{, K}^+]$ in DMF at 295 K. Experimental in black and simulated in red.
Simulation parameters: $g = 2.00185$

1) Ring protons (8): -1.20G/-0.17G
2) Sidechain $\alpha$ protons (4): 0.52G/0.07G
3) Nitrogen atoms (2): 0.15G/-0.21G
4) Linewidth: 0.23G

**Figure S7.** EPR spectrum of [PEG$_8$–PDI$^-$, K$^+$] in DMF at 260 K. Experimental in black and simulated in red.
**Figure S8.** Temperature dependence of the EPR intensity ($I_{EPR}$) of the PEG$_8$–PDI$^-$ in DMF.

**Figure S9.** Structure of PD1-1.$^{S11}$
Figure S10. LSV curves of RDEVs of bare glassy carbon and PDI-1 in O$_2$-saturated 0.1 M NaHPO$_4$/NaH$_2$PO$_4$ buffer solution at pH 7 with rotating speed of 1600 rpm.

Figure S11. Electrochemical stability measurements of the PDI-1 after 1000 cycles in O$_2$-saturated 0.1 M NaHPO$_4$/NaH$_2$PO$_4$ buffer solution at pH 7 with rotating speed of 1600 rpm.
The description of the disorder refinement in crystal structure of the PEG₃−PDI⁻ anion. In the process of structure refinement of PEG₃−PDI⁻ anion, it was found that the O(4), C(22), C(23), O(5) and C(24) atoms (Figure S13) of PEG₃ group have enlarged displacement ellipsoids. That was attributed to the conformational disorder of the PEG₃ group near the atom C(21). We refined the occupations of the two positions of atoms O(4), C(22), C(23), O(5), C(24) and O(4’), C(22’), C(23’), O(5’), C(24’) corresponding to two different PEG₃-group conformations. Two positions of the PEG₃-group were successfully modeled; they were refined with the help of similarity restraints on C-C (1.510(1)Å) and C-O (1.430(1)Å) bond length and displacement parameters, as well as equal restraints for anisotropic displacement parameters of the disordered atoms. After that the hydrogen atoms were added to the calculated positions of the disordered carbons and refined as a rigid model. The relative occupancies for the disordered components were refined freely, while constraining the total occupancy of all components to unity. As a result of the refinement we obtained relative occupancies equal to 73.7% and 26.3% for these two positions.

Additional details of structure refinement and justification for publishing. At the end of the structure refinement, the [PEG₃−PDI⁻ Cp₂Co⁺] crystal analysis produced quite elevated R-factors values (Table S1). In addition, there a 2.07 eÅ⁻³ peak in the Fourier difference density map was found. This peak is located ~2.4 Å from the Co-atom and probably cannot be an atom. This effect is probably due to the twinning of the crystal. However, when we calculated a TWIN matrix for this crystal using ROTAX¹² software and applied it in this refinement, no significant improvements of the structural parameters were observed. Even with the limited data, the publication of this structure in its current state was justified due to the very complex procedure of crystallizing this material. Hence, we cannot provide a detailed molecular geometry of this
material; however, the result of this experiment can be used to present the qualitative structure of this material and compare it with structures of other complexes.

**Table S1.** Selected crystal data and details on the structure determination from the single crystal data for [PEG$_3$−PDI$^-$ Cp$_2$Co$^+$]

| compound | [PEG$_3$−PDI$^-$ Cp$_2$Co$^+$] |
|----------|-------------------------------|
| formula  | C$_{48}$H$_{48}$CoN$_2$O$_{10}$ |
| crystal color | dark-green                  |
| MW [g·mol$^{-1}$] | 871.81                     |
| crystal system | monoclinic                 |
| space group | C $2/c$                     |
| $a$ [Å]    | 28.11(3)                    |
| $b$ [Å]    | 17.578(16)                  |
| $c$ [Å]    | 8.427(8)                    |
| $\beta$ [deg] | 102.328(11)                |
| $V$ [Å$^3$] | 4069(6)                     |
| $T$ [K]    | 173(2)                      |
| $Z$        | 4                            |
| $D_{\text{calc}}$ [g·cm$^{-3}$] | 1.423                     |
| $\mu$ [mm$^{-1}$] | 0.487                    |
| $\theta_{\text{max}}$ [deg] | 26.00                     |
| measured reflections | 6433                     |
| unique reflections | 3800                      |
reflections [I > 2σ(I₀)] 1488
parameters 270
restrains 18
$R_{int}$ 0.0960
$R_1$ [I > 2σ(I₀)] 0.1252
$wR_2$ [all data] 0.3149
GOF 1.006
$\Delta\rho_{max}/\Delta\rho_{min}$ [e·Å⁻³] 2.07/-0.41

**Figure S12.** Asymmetric unit of PEG₃–PDI⁻ anion drawn at 50% ellipsoidal probability level.
References:

S1. Kohmoto, S.; Mori, E.; Kishikawa, K. Room-Temperature Discotic Nematic Liquid Crystals over a Wide Temperature Range: Alkali-Metal-Ion-Induced Phase Transition from Discotic Nematic to Columnar Phases. *J. Am. Chem. Soc.* **2007**, *129*, 13364–13365.

S2. Vacca, J. P.; Dorsey, B. D.; Guare, J. P.; Holloway, K. M.; Hungate, R. W.; Levin, R. B. U.S. Patent 5,413,999, issued May 9, 1995.

S3. Dombi, K. L.; Griesang, N.; Richert, C. Oligonucleotide Arrays from Aldehyde-Bearing Glass with Coated Background. *Synthesis* **2002**, *6*, 816–824.

S4. Schmidt, M.; Amstutz, R.; Crass, G.; Seebach, D. Herstellung Einiger Chiraler Aminodiole aus Weinsäure. Chirale Lithiumalanate für Asymmetrische Hydrierungen. *Chem. Ber.* **1980**, *113*, 1691–1707.

S5. Ouchi, M.; Inoue, Y.; Liu, Y.; Nagamune, S.; Nakamura, S.; Wada, K.; Hakushi, T. Convenient and Efficient Tosylation of Oligoethylene Glycols and the Related Alcohols in Tetrahydrofuran–Water in the Presence of Sodium Hydroxide. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1260–1262.

S6. Sayaka, M.; Iwamoto, H.; Mizutani, T. Water Accessibility to the Binding Cleft as a Major Switching Factor from Entropy-Driven to Enthalpy-Driven Binding of an Alkyl Group by Synthetic Receptors. *Chem. Asian J.* **2010**, *5*, 1163–1170.

S7. Thomas, J. D.; Burke, T. R. Application of a Water-Soluble Pyridyl Disulfide Amine Linker for use in Cu-Free Click Bioconjugation. *Tet. Lett.* **2011**, *52*, 4316–4319.

S8. Sayaka, M.; Iwamoto, H.; Mizutani, T. Water Accessibility to the Binding Cleft as a Major Switching Factor from Entropy-Driven to Enthalpy-Driven Binding of an Alkyl Group by Synthetic Receptors. *Chem. Asian J.* **2010**, *5*, 1163–1170.
S9. Ramakrishna, S.; Zhang, X.; Wadkins, R. W.; Mattern, D. L. Synthesis of a Non-Cationic, Water-Soluble Perylenetetracarboxylic Diimide and its Interactions with G-Quadruplex-Forming DNA. *J. Bioorg. Med. Chem.* **2007**, *15*, 186–193.

S10. Ryu, J.-H.; Jang, C-J.; Yoo, Y-S.; Lim, S-G.; Lee, M. Supramolecular Reactor in an Aqueous Environment: Aromatic Cross Suzuki Coupling Reaction at Room Temperature. *J. Org. Chem.* **2005**, *70*, 8956–8962.

S11. Chen, S.; Liu, Y.; Qiu, W.; Sun, X.; Ma, Y.; Zhu, D. Oligothiophene-Functionalized Perylene Bisimide System: Synthesis, Characterization, and Electrochemical Polymerization Properties. *Chem. Mater.* **2005**, *17*, 2208–2215.

S12. Cooper, R. I.; Gould, R. O.; Parsons, S.; Watkin, D. J. The Derivation of Non-Merohedral Twin Laws During Refinement by Analysis of Poorly Fitting Intensity Data and the Refinement of Non-Merohedrally Twinned Crystal Structures in the Program CRYSTALS. *J. Appl. Cryst.* **2002**, *35*, 168–174.