Biomaterials Science

Hemoglobin-mediated synthesis of PEDOT:PSS: Enhancing conductivity through biological oxidants

Journal:	*Biomaterials Science*
Manuscript ID:	BM-ART-09-2014-000338.R3
Article Type:	Communication
Date Submitted by the Author:	02-Jan-2015
Complete List of Authors:	Morris, Joshua; Georgia Gwinnett College, Khanal, Dipesh; Georgia Institute of Technology, Richey, Jessica; Georgia Institute of Technology, Payne, Christine; Georgia Institute of Technology,

Biomaterials Science

RSCPublishing

COMMUNICATION

Cite this: DOI: 10.1039/x0xx00000x Hemoglobin-mediated synthesis of PEDOT:PSS: Enhancing conductivity through biological oxidants

J.D. Morris,[b] D. Khanal,[a] J. A. Richey,[a] and C. K. Payne*[a]

Received 00th January 2015,
Accepted 00th January 2015

DOI: 10.1039/x0xx00000x

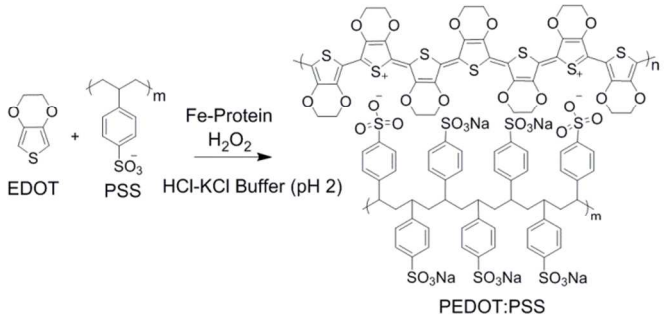
www.rsc.org/

Hemoglobin- and catalase-polymerized PEDOT:PSS were characterized by X-ray photoelectron spectroscopy, visible and near-IR spectroscopy, FTIR, and ESR. Hemoglobin-polymerized PEDOT:PSS possesses bipolarons, while catalase-polymerized PEDOT:PSS is dominated by polarons. Use of heme-bound iron as an oxidant yields PEDOT:PSS with conductivity of 19.5 S cm^{-1} in a single-step aqueous reaction.

Introduction

Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) is a transparent, water dispersible, and biocompatible conductive polymer mixture.[1, 2] These properties enable the use of PEDOT:PSS in a diverse set of applications including light emitting diodes,[3-6] chemical and biological sensors,[7-10] and regenerative medicine.[11, 12] The initial synthesis of PEDOT:PSS results in a relatively low conductivity polymer (~1 S cm^{-1}).[1] Conductivity is then enhanced by treating films of low-conductivity PEDOT:PSS with organic molecules, inorganic salts, or inorganic acids.[13-16] This process increases the conductivity of PEDOT:PSS from ~1 S cm^{-1} up to ~3000 S cm^{-1}.[17] To streamline production, there is a significant advantage to controlling the properties of PEDOT:PSS during polymerization. This can be accomplished by the choice of oxidant used during polymerization.[18, 19] Recently, we demonstrated a six order of magnitude increase in the conductivity of PEDOT:PSS as a function of oxidant. Specifically, the use of hemin as an oxidant led to PEDOT:PSS films with conductivities of 0.62 S cm^{-1}, compared to films produced using $FeCl_3$ as an oxidant with conductivities of 2.9x10^{-7} S cm^{-1}.[18] We found that this increase in conductivity was associated with a change in the dominant charge carrier species. Hemin-synthesized PEDOT:PSS possessed bipolarons, while $FeCl_3$-synthesized PEDOT:PSS possessed polarons. In addition to our work with PEDOT:PSS, oxidants such as $Fe_2(SO_4)_3$, $(NH_4)_2S_2O_8$, and H_2O_2 have been used to tune the conductivity of a similar conducting polymer, poly(thieno[3,4-b]thiophene), over two orders of magnitude.[19]

While small molecule oxidants provide a measure of control over the final polymer conductivity, biomolecules provide a broad array of properties and could serve as an oxidant library for the synthesis of conducting polymers with a desired set of properties. Initial work in the use of proteins as oxidants for the synthesis of PEDOT:PSS focused on plant enzymes,[20-25] specifically horseradish and soybean peroxidase.[23, 25] We have previously shown that both active and denatured catalase can be used as oxidants for the polymerization of PEDOT:PSS.[26] Enzymatic activity is not required for polymerization. Instead, catalase serves only as a source of iron. Our current research investigates the effect of protein oxidant on polymer properties using two iron-containing biomolecules, hemoglobin (Hb) and catalase (CAT) (Fig. S1), as oxidants for the polymerization of PEDOT:PSS (Scheme 1). We show that different heme proteins result in dramatically different polymer conductivities and investigate the mechanistic underpinnings of this difference.



Scheme 1. Reaction scheme using an iron-containing protein as an oxidant for the synthesis of PEDOT:PSS. Hb and CAT were used as oxidants (Fig. S1, ESI).

PEDOT:PSS was synthesized by combining the appropriate biological oxidant (Hb or CAT), EDOT, and PSS (70 kDa MW) in an HCl-KCl buffer (pH ~2) while stirring. The reaction was initiated by the addition of stoichiometric quantities of hydrogen peroxide. The reaction was allowed to proceed for 6 hours. The product was then isolated through dialysis and centrifugation. Elemental analysis by XPS shows that the polymers have nearly identical elemental compositions and that protein is not present in the final product (Table S1 and Fig. S2, ESI). Complete experimental details can be found in ESI. Interestingly, the polymerization of PEDOT:PSS by Hb and CAT yields polymers with differing optical and electronic properties (Fig. 1).

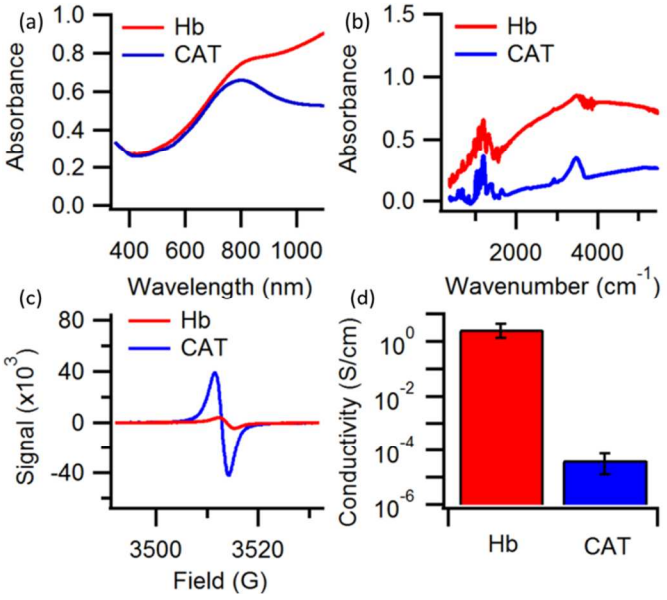


Fig. 1. Spectroscopic and electrical characterization of PEDOT:PSS polymerized with Hb and CAT. (a) Representative visible and near IR absorption spectra of Hb-synthesized PEDOT:PSS (red) and CAT-synthesized PEDOT:PSS (blue). (b) Representative FTIR spectra. (c) Representative ESR spectra. (d) Conductivity of Hb-synthesized PEDOT:PSS films (red) and CAT-synthesized PEDOT:PSS films (blue) measured by four point probe. Error bars represent the standard deviation of a minimum four measurements on twelve films.

Visible and near IR absorption spectra of Hb- and CAT-polymerized PEDOT:PSS both show a strong absorption at 795 nm characteristic of polarons (Fig. 1a). Hb-polymerized PEDOT:PSS shows increasing absorption in the near IR suggesting a peak further into the IR. This was confirmed with FTIR measurements in the mid-IR that showed a broad absorption centered at 3600 cm^{-1} (Fig. 1b) for Hb-polymerized PEDOT:PSS. This feature has been previously attributed to bipolarons in PEDOT:PSS.[27] CAT-polymerized PEDOT:PSS lacks this bipolaron absorption. This difference in spectral features suggests that CAT-polymerized PEDOT:PSS primarily possesses polarons, while Hb-polymerized PEDOT:PSS possesses a mixture of polarons and bipolarons. This conclusion is supported by the ESR spectra of Hb- and CAT-polymerized PEDOT:PSS (Fig. 1c). ESR is a useful tool for characterizing polarons as only unpaired charges, such as the radical cations of polarons, result in absorption. Paired species, such as bipolarons, are not observed with ESR.[28-31] CAT-polymerized PEDOT:PSS shows a strong absorption centered at 3512 G, while Hb-polymerized PEDOT:PSS shows a much weaker absorption at 3513 G. This change in absorption intensity indicates that CAT-polymerized PEDOT:PSS possesses a significantly higher concentration of polarons than Hb-polymerized PEDOT:PSS. This difference in charge carrier species should be reflected in different conductivities with the higher charge density provided by bipolarons leading to increased conductivity.[2] The conductivity of Hb- and CAT-polymerized PEDOT:PSS was measured using a four point probe (Fig. 1d). We find the Hb-polymerized PEDOT:PSS possess a conductivity of 2.8 S cm^{-1}, compared to $3.5x10^{-5}$ S cm^{-1} for CAT-polymerized PEDOT:PSS. The use of myoglobin, which is structurally similar to Hb, as an oxidant also resulted in bipolarons as the dominant charge carrier (Fig. S3, ESI). While the conductivity of the Hb-polymerized PEDOT:PSS is not especially high, the important aspect of this result is the ability to synthesize PEDOT:PSS with conductivities spanning five orders of magnitude in a single-step aqueous reaction by the choice of biomolecular oxidant.

Although the iron in both Hb and CAT is associated with identical heme B groups, they yield PEDOT:PSS with different charge carrier species and different conductivities suggesting the difference in polymer properties arises from a difference in the protein. It is important to note that these reactions are carried out under acidic conditions (pH ~2) where the proteins are at least partially denatured. Catalase has minimal enzyme activity (Fig. S4, ESI) and Hb shows spectral shifts characteristic of the acid denaturation of Fe(III) methemoglobin (Fig. S5, ESI). The observed difference in polymer properties can be compared to our previous work with $FeCl_3$-polymerized PEDOT:PSS, which possesses polarons, and hemin-polymerized PEDOT:PSS, which possesses bipolarons.[18] Hemin and heme B differ only in the initial oxidation state of iron, Fe(III) and Fe(II), respectively, and the presence of a chloride counter ion. We hypothesized that protein structure, even denatured protein structure, controls polymer properties through differing rates of the release of iron into solution from the heme group.

To test this hypothesis, an iron chelator, ethylenediaminetetraacetic acid (EDTA), was added to the

reaction mixture to remove free iron from the reaction. As a control, EDTA was added to a reaction using $FeCl_3$ as the oxidant, a reaction that can proceed only through free iron. No PEDOT:PSS was formed (Fig. S6, ESI). In comparison, hemin-mediated polymerization of PEDOT:PSS was not affected by EDTA (Fig. S6, ESI). Using CAT as an oxidant, the addition of EDTA reduced the yield of PEDOT:PSS by a factor of 20 (Fig. 2a). Importantly, the presence of EDTA in the reaction mixture shifts the charge carrier in the resulting PEDOT:PSS from polarons to bipolarons (Fig. 2). Visible and near IR spectra show enhanced absorption at longer wavelengths, indicative of bipolarons (Fig. 2a). Similarly, the FTIR spectra of PEDOT:PSS polymerized by CAT in the presence of EDTA shows a broad absorption feature due to bipolarons (Fig. S7, ESI). The ESR spectra of CAT-polymerized PEDOT:PSS with EDTA demonstrates a dramatically reduced concentration of polarons compared to the same amount of PEDOT:PSS polymerized in the absence of EDTA (Fig. 2b).

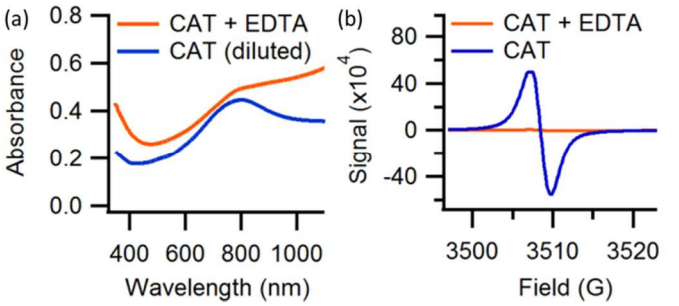


Fig. 2. Spectroscopic characterization of PEDOT:PSS synthesized with CAT in the presence (orange) and absence (blue) of EDTA. (a) Representative visible and near IR absorption spectra. CAT in the absence of EDTA is replotted from Fig. 1 for comparison. CAT (blue) is diluted 20 fold compared to CAT + EDTA (orange). (b) Representative ESR spectra. FTIR spectra show similar trends (Fig. S7, ESI).

In comparison, Hb-polymerized PEDOT:PSS was minimally affected by the addition of EDTA (Fig. S8, ESI). The yield was reduced by less than a factor of 3 (Fig. S8a, ESI). The reduced yield demonstrates that polymerization mediated by both Hb and CAT is due to a mixture of heme B, which is not affected by EDTA, and free iron, which is inhibited by EDTA. In the presence of EDTA, the ESR spectra of Hb-polymerized PEDOT:PSS shows a decrease in the already weak polaron absorption (Fig. S8b, ESI). For both Hb and CAT, the reduction in polaron population is due to the reduced availability of free iron during PEDOT:PSS polymerization. These results demonstrate that the primary difference between Hb- and CAT-polymerized PEDOT:PSS arise from a difference in active iron oxidant. When heme B is the active oxidant, bipolarons dominate. When free iron is the active oxidant, polarons dominate.

Given the conductivity enhancement associated with the change in charge carrier species from polaron to bipolarons (Fig. 1d), we expect a further enhancement for PEDOT:PSS polymerized by Hb in the presence of EDTA, which only allows for heme B, rather than free iron, mediated polymerization. The conductivity of Hb-polymerized PEDOT:PSS in the presence of EDTA was measured to be 19.5 S cm^{-1}, a nearly 10 fold enhancement compared to Hb-polymerized PEDOT:PSS in the absence of EDTA. To the best of our knowledge, this enhanced conductivity represents the highest on record for pristine PEDOT:PSS, which is typically < 1 S cm^{-1}.[2, 32, 33] The highest previously reported conductivity for pristine PEDOT:PSS was 10 S cm^{-1}, obtained by creating an emulsion of the monomer EDOT in PSS before polymerization for higher concentrations of EDOT an aqueous solution.[34] Hb-polymerized PEDOT:PSS (with EDTA) is considerably more conductive than PEDOT:PSS previously obtained via biological oxidants, with conductivities between 10^{-5} to 10^{-3} S cm^{-1}.[22, 23, 25, 26] In the case of horseradish peroxidase and soybean peroxidase, which are both heme B proteins, it is likely that free iron also contributed to the polymerization, resulting in low conductivities.[23, 25, 35]

Conclusions

We show that Hb-polymerized PEDOT:PSS is 10^5 times more conductive than catalase polymerized PEDOT:PSS. This difference in conductivity is correlated with a change in charge carrier species, confirmed with near-IR absorption, mid-IR absorption, and electron spin resonance (ESR) spectroscopy. Hb-synthesized PEDOT:PSS possesses bipolarons while CAT-synthesized PEDOT:PSS possesses polarons (Fig. 1). Mechanistic studies show that this difference in charge carrier species is ultimately controlled by the dominant oxidant: heme iron is the dominant oxidant in the Hb synthesis, while free iron is the dominant oxidant in the CAT synthesis (Fig. 2). When free iron is removed from the polymerization by chelation with EDTA, the resulting PEDOT:PSS is dominated by bipolarons with a conductivity of 19.5 S cm^{-1}.

The key difference between the use of heme B and free iron as an oxidant is likely due to the interaction with hydrogen peroxide. It is known that Hb and CAT can form high-valent iron species when combined with hydrogen peroxide.[36, 37] For example, the biological function of active CAT is the decomposition of hydrogen peroxide via the formation of a high-valent iron species known as compound I.[38] Previous investigations of Hb have shown that it can form oxoiron(IV) following reaction with hydrogen peroxide.[39, 40] In addition, production of hydroxyl radicals via Fenton's reaction may mediate the reaction.[41, 42] Future work will examine the interaction between the iron-containing biomolecules and hydrogen peroxide to characterize the active oxidant in the polymerization.

Using EDTA allows for a single-step aqueous reaction yielding PEDOT:PSS with relatively high conductivity. Although this conductivity remains lower than the record for PEDOT:PSS, the processing involved is considerably reduced. The highest conductivities on record are achieved through sequential processing steps.[43] For example, when PEDOT:PSS is treated with ethylene glycol before and after film casting, conductivities as high as 1400 S cm^{-1} can be achieved. PEDOT:PSS films treated multiple times with sulfuric acid

reach the highest conductivities on record of over 3000 S cm^{-1} [17].

The use of iron-containing biomolecules as oxidants for the synthesis of PEDOT:PSS dramatically expands the structures of oxidants that can be screened for potentially valuable PEDOT:PSS properties. There are ~800 different heme proteins catalogued in the heme protein database.[35] Obtaining such diversity synthetically would be a difficult undertaking. The naturally occurring array of iron-containing proteins provides a readymade set of highly specialized oxidants. These diverse structures provide a means of controlling PEDOT:PSS properties in a single-step aqueous reaction yielding a tailor-made polymer with the appropriate conductivity for a given application.

Acknowledgements

The authors thank Prof. Jake Soper for helpful comments and use of the FTIR, Prof. Jiri Janata for use of the four point probe station, Dr. Robert Braga for help with the ESR, and Timothy Atallah for helpful discussion. This work was supported by a DARPA Young Faculty Award (N66001-11-1-4126) to CKP.

Notes and references

[a] School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, United States and Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[b] School of Science and Technology, Georgia Gwinnett College, Lawrenceville, Georgia 30043, United States

†Electronic Supplementary Information (ESI) available: Complete experimental details, protein structures, characterization of myoglobin polymerized PEDOT:PSS, CAT activity assay, Hb spectral analysis, characterization of $FeCl_3$- and hemin-polymerized PEDOT:PSS in the presence of EDTA, and characterization of Hb-polymerized PEDOT:PSS with EDTA,. See DOI: 10.1039/b000000x/

1 B. L. Groenendaal, F. Jonas, D. Freitag, H. Pielartzik and J. R. Reynolds, *Adv. Mater.*, 2000, **12**, 481-494.
2 S. Kirchmeyer and K. Reuter, *J. Mater. Chem.*, 2005, **15**, 2077-2088.
3 W. H. Kim, A. J. Makinen, N. Nikolov, R. Shashidhar, H. Kim and Z. H. Kafafi, *Appl. Phys. Lett.*, 2002, **80**, 3844-3846.
4 M.-C. Choi, Y. Kim and C.-S. Ha, *Prog. Polym. Sci.*, 2008, **33**, 581-630.
5 C. Zhong, C. Duan, F. Huang, H. Wu and Y. Cao, *Chem. Mater.*, 2010, **23**, 326-340.
6 C. A. Zuniga, S. Barlow and S. R. Marder, *Chem. Mater.*, 2011, **23**, 658-681.
7 J. A. Arter, D. K. Taggart, T. M. McIntire, R. M. Penner and G. A. Weiss, *Nano Lett.*, 2010, **10**, 4858-4862.
8 K. C. Donavan, J. A. Arter, R. Pilolli, N. Cioffi, G. A. Weiss and R. M. Penner, *Anal. Chem.*, 2011, **83**, 2420-2424.
9 Y. Cao, A. E. Kovalev, R. Xiao, J. Kim, T. S. Mayer and T. E. Mallouk, *Nano Lett.*, 2008, **8**, 4653-4658.
10 N. K. Guimard, N. Gomez and C. E. Schmidt, *Prog. Polym. Sci.*, 2007, **32**, 876-921.
11 S. M. Richardson-Burns, J. L. Hendricks and D. C. Martin, *J. Neural. Eng.*, 2007, **4**, L6-L13.
12 S. M. Richardson-Burns, J. L. Hendricks, B. Foster, L. K. Povlich, D. H. Kim and D. C. Martin, *Biomaterials*, 2007, **28**, 1539-1552.
13 R. Po, C. Carbonera, A. Bernardi, F. Tinti and N. Camaioni, *Sol. Energy Mater. Sol. Cells*, 2012, **100**, 97-114.
14 J. Ouyang, Q. F. Xu, C. W. Chu, Y. Yang, G. Li and J. Shinar, *Polymer*, 2004, **45**, 8443-8450.
15 J. Y. Kim, J. H. Jung, D. E. Lee and J. Joo, *Synth. Met.*, 2002, **126**, 311-316.
16 J. Ouyang, C. W. Chu, F. C. Chen, Q. Xu and Y. Yang, *Adv. Funct. Mater.*, 2005, **15**, 203-208.
17 Y. Xia, K. Sun and J. Ouyang, *Adv. Mater.*, 2012, **24**, 2436-2440.
18 J. D. Morris and C. K. Payne, *Org. Electron.*, 2014, **15**, 1707-1710.
19 B. Lee, V. Seshadri and G. A. Sotzing, *Langmuir*, 2005, **21**, 10797-10802.
20 S. Kobayashi, *J. Polym. Sci., Part A-1: Polym. Chem.*, 1999, **37**, 3041-3056.
21 S. Kobayashi, H. Uyama and S. Kimura, *Chem. Rev.*, 2001, **101**, 3793-3818.
22 G. Shumakovich, G. Otrokhov, I. Vasil'eva, D. Pankratov, O. Morozova and A. Yaropolov, *J. Mol. Catal. B: Enzym.*, 2012, **81**, 66-68.
23 V. Rumbau, J. A. Pomposo, A. Eleta, J. Rodriguez, H. Grande, D. Mecerreyes and E. Ochoteco, *Biomacromolecules*, 2007, **8**, 315-317.
24 A. Tewari, A. Kokil, S. Ravichandran, S. Nagarajan, R. Bouldin, L. A. Samuelson, R. Nagarajan and J. Kumar, *Macromol. Chem. Phys.*, 2010, **211**, 1610-1617.
25 S. Nagarajan, J. Kumar, F. F. Bruno, L. A. Samuelson and R. Nagarajan, *Macromolecules*, 2008, **41**, 3049-3052.
26 S. M. Hira and C. K. Payne, *Synth. Met.*, 2013, **176**, 104-107.
27 F. L. E. Jakobsson, X. Crispin, L. Lindell, A. Kanciurzewska, M. Fahlman, W. R. Salaneck and M. Berggren, *Chem. Phys. Lett.*, 2006, **433**, 110-114.
28 A. Zykwinska, W. Domagala, A. Czardybon, B. Pilawa and M. Lapkowski, *Chem. Phys.*, 2003, **292**, 31-45.
29 R. Jalili, J. M. Razal, P. C. Innis and G. G. Wallace, *Adv. Funct. Mater.*, 2011, **21**, 3363-3370.
30 J. Cornil and J.-L. Brédas, *Adv. Mater.*, 1995, **7**, 295-297.
31 Y. Furukawa, *J. Phys. Chem.*, 1996, **100**, 15644-15653.
32 J. Y. Kim, J. H. Jung, D. E. Lee and J. Joo, *Synth. Met.*, 2002, **126**, 311-316.
33 M. Reyes-Reyes, I. Cruz-Cruz and R. n. López-Sandoval, *J. Phys. Chem. C*, 2010, **114**, 20220-20224.
34 Z. Qi and P. G. Pickup, *Chem. Commun.*, 1998, 2299-2300.
35 C. J. Reedy, M. M. Elvekrog and B. R. Gibney, *Nucleic Acids Res.*, 2008, **36**, D307-313.
36 D. A. Svistunenko, *Biochim. Biophys. Acta*, 2005, **1707**, 127-155.
37 D. A. Svistunenko, *Biochim. Biophys. Acta*, 2001, **1546**, 365-378.
38 P. Gouet, H. M. Jouve, P. A. Williams, I. Andersson, P. Andreoletti, L. Nussaume and J. Hajdu, *Nat. Struct. Biol.*, 1996, **3**, 951-956.
39 J. F. Gibson and D. J. E. Ingram, *Nature*, 1956, **178**, 871-872.
40 E. Nagababu and J. M. Rifkind, *Biochemistry*, 2000, **39**, 12503-12511.
41 J. Prousek, in *Pure Appl. Chem.*, 2007, vol. 79, p. 2325.
42 A. Puppo and B. Halliwell, *Biochem. J*, 1988, **249**, 185-190.
43 Y. H. Kim, C. Sachse, M. L. Machala, C. May, L. Müller-Meskamp and K. Leo, *Adv. Funct. Mater.*, 2011, **21**, 1076-1081.

