The electrochemically-tuneable interactions between flavin-functionalised C$_{60}$ derivatives and 2,6-diethylamidopyridine†

Joseph B. Carroll,[a] **Graeme Cooke,‡**[*b] **James F. Garety,**[b] **Brian J. Jordan,**[a] **Suhil Mabruk**[b] **and Vincent M. Rotello**[a]

We report the electrochemically-tuneable interactions between flavin-functionalised C$_{60}$ derivatives and a diamidopyridine derivative.

The electrochemically controllable hydrogen bonding interactions between flavin and complementary diamidopyridine derivatives are well documented, and have paved the way for the development of solution-based models for probing flavoenzyme activity and the creation of biologically inspired molecular devices.[1] More recently, the electrochemically-tuneable interactions between flavin units and diamidopyridine moieties immobilised onto gold nanoparticles have been reported.[2] However, due to the poorly defined and fairly inaccessible electrochemical properties of the gold core, the role the gold substrate plays in modulating the redox and molecular recognition properties of the flavin has not been reported. Buckminsterfullerene (C$_{60}$), by virtue of its three-dimensional structure and interesting electrochemical[3] and optical properties, has become an important building block in supramolecular,[4] materials[5] and biological chemistry.[6] The rich synthetic chemistry displayed by this carbon-based sphere,[7] could provide a new platform for synthesising the next generation of immobilised flavin derivatives. In addition, the redox behaviour of the C$_{60}$ moiety could be exploited as a means of modulating the redox properties and host–guest complexation of the flavin unit. Here we report the synthesis and electrochemical properties of flavin-functionalised C$_{60}$ derivatives **1** and **2** and their subsequent electrochemically-tuneable interactions with 2,6-diethylamidopyridine **3**.

Compounds **1**, **2** and **4** were synthesised from C$_{60}$, sarcosine and the corresponding aldehyde using a previously reported methodology†.[8] An interesting feature of flavin-functionalised systems is the visible quenching of the flavin fluorescence by the C$_{60}$ moiety when irradiated with light of $\lambda = 365$ nm. Interestingly, very little difference in the ^{1}H NMR spectra of **1** and **2** were observed when they were compared to the spectra of 7,8-dimethyl-10-isobutyl flavin **5** and 7-trifluoromethyl-10-isobutyl flavin **6**

[a]*Department of Chemistry, University of Massachusetts at Amherst, Amherst, MA 01002, USA*
[b]*Centre for Biomimetic Design & Synthesis, Chemistry, School of Engineering & Physical Sciences, Heriot-Watt University, Riccarton, Edinburgh, UK, EH14 4AS. E-mail: g.cooke@hw.ac.uk*
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‡ Correspondence address from 1st October 2005: Dr. Graeme Cooke, Department of Chemistry, Joseph Black Building, University of Glasgow, University Avenue, Glasgow, UK G12 8QQ. E-mail: g.cooke@chem.gla.ac.uk
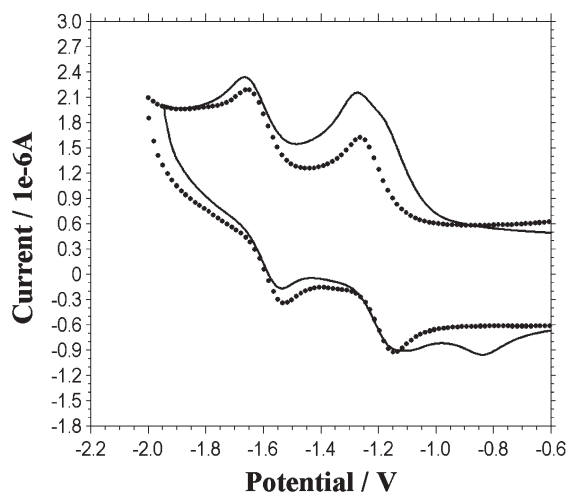

1 R$_1$ = R$_2$ = CH$_3$
2 R$_1$ = H, R$_2$ = CF$_3$

5 R$_1$ = R$_2$ = CH$_3$
6 R$_1$ = H, R$_2$ = CF$_3$

(CDCl$_3$) respectively, indicating rather limited interactions occur between these units in their neutral states.

The solution electrochemistry of **1**, **2** and **4** (0.1 M Bu$_4$NPF$_6$ in CH$_2$Cl$_2$) have been studied using cyclic voltammetry (CV) and square wave voltammetry (SWV).[9] Upon reduction of flavin-functionalised derivatives **1** and **2**, overlapping redox waves corresponding to the reduction of the C$_{60}$ and flavin units were observed (Fig. 1).[10] However, by performing SWV on these derivatives, it was possible to resolve the reduction waves of these systems into fairly distinct waveforms. For both flavin derivatives, a single reduction wave (confirmed by second-derivative SWV†) was observed for the pseudoreversible formation of the flavin radical anion species **1**$_{\mathbf{rad}}$$^-$ ($E_{1/2} = \sim -1.32$ V) and **2**$_{\mathbf{rad}}$$^-$ ($E_{1/2} = \sim -1.15$ V). A second reoxidation wave for **2** ($E = -0.84$ V) arises from an electrochemical–chemical–electrochemical (e–c–e) process, where a portion of **2**$_{\mathbf{rad}}$$^-$, formed at the electrode surface, rapidly deprotonates **2** in the bulk medium†.[11] The protonated flavin radical (**2**$_{\mathbf{rad}}$H) produced in this process undergoes a further one-electron reduction at the working electrode surface to form the relatively stable fully reduced flavin anion (**2**$_{\mathbf{red}}$H$^-$), which is subsequently reoxidised at a less negative potential than **2**$_{\mathbf{rad}}$$^-$. The second reoxidation wave for **1** is presumably masked by the first reduction wave of the C$_{60}$ moiety. As anticipated, the disparate electronic properties of the functional groups attached to the 7- and 8-positions of the flavin, had a major role in determining the redox properties of the flavin units of **1** and **2**.[12] In particular, the redox waves due to the formation of **1**$_{\mathbf{rad}}$$^-$ and **2**$_{\mathbf{rad}}$$^-$ occurred at a more and a less negative potential, respectively, than the redox wave due to the first reduced state of the C$_{60}$ unit. The CV of compound **4** gave rise to two redox waves

Fig. 1 CVs of **2** (—) and **4** (......) (~1 × 10^{-4} M). Scan rate = 500 mV s^{-1}.

at −1.21 V and −1.59 V, corresponding to the formation of **4**$^-$ and **4**$^{2-}$, respectively.[3]

To evaluate the effect the juxtaposition of the redox-active flavin and C$_{60}$ moieties have on their respective electrochemical properties, we compared the CV and SWV data for derivatives **1** and **2** with those of compounds **4**, **5** and **6**†. For derivative **1**, the attachment of the flavin unit to the C$_{60}$ moiety shifts the redox waves of the latter to more positive potentials (typically +30 mV), indicating that the flavin unit facilitates the formation of the first two reduced states of the C$_{60}$ unit of this derivative. Similar data were observed for **2**, however, in this case, the positive shift in the redox waves for the C$_{60}$ moieties were significantly less than those observed for **1** (typically +10 mV). When the effect the attachment of C$_{60}$ has on the redox behaviour of the flavin moiety was investigated by comparing electrochemical data of **1** or **2** to that obtained for **5** or **6** (see ESI† and Fig. 2), it was observed that in both cases, the C$_{60}$ moiety shifts the redox wave due to formation of the flavin radical anion state to more positive potentials (typically +30 to +60 mV). Therefore, it is apparent that the covalent attachment of flavin units to C$_{60}$ (and *vice versa*) results in the stabilisation of the reduced states of these species.
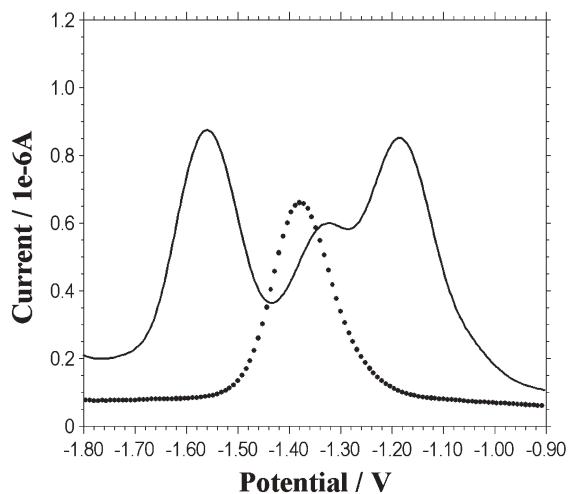

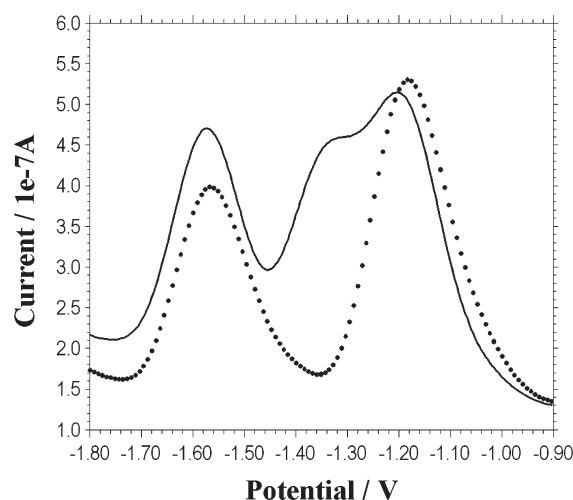**Fig. 2** SWVs of **1** (—) and **5** (......) (~0.8 × 10^{-4} M).

Fig. 3 SWVs of **1** (~0.5 × 10^{-4} M) before (—) and after (......) the addition of excess **3** (~ 4 × 10^{-2} M).

With the electrochemical properties of the individual flavin-functionalised C$_{60}$ derivatives investigated, we next focused our attention onto determining whether the redox controllable recognition properties of the flavin unit can be exploited. Rotello and co-workers have previously shown that intermolecular hydrogen bonding interactions between **3** and complementary **5** results in a +150 mV shift in the half-wave potential of the flavin radical anion (in CH$_2$Cl$_2$), indicating that a significant stabilisation of this state occurs.[13] Moreover, the shift in redox potential corresponds to a 500-fold increase in the binding efficiency of the host–guest complex upon electrochemical reduction of the flavin unit.

In order to determine whether similar redox controllable complexation properties can be achieved between derivatives **1** or **2** and **3**, we investigated the addition of an excess of compound **3** to separate electrolyte solutions of **1** or **2**. The redox waves corresponding to the **1/1$_{rad}$**$^-$ or **2/2$_{rad}$**$^-$ redox couples were immediately shifted by +110 mV (see Fig. 3, Fig. 4 and ESI†), corresponding to a substantial stabilisation of the flavin radical anion by 11 kJ mol^{-1}, consistent with more than a 70-fold increase
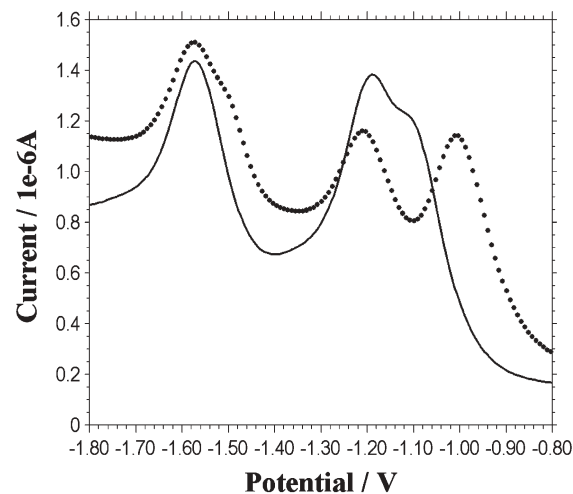

Fig. 4 SWVs of **2** (~1 × 10^{-4} M) before (—) and after (......) the addition of excess **3** (~ 2 × 10^{-2} M).

in the binding strength of the complex (3.1_{rad}^-: K_a = 39143 ± 1960 M^{-1}, 3.2_{rad}^-: K_a = 27907 ± 1395 M^{-1}).[14] In particular, upon the addition of an excess of **3**, the flavin radical anion redox wave for derivative **1** now coincides with the redox wave for the first reduced state of C_{60}. In contrast, this redox wave for the flavin moiety of **2**, which originally occurred at a more positive potential than the first reduction wave of the C_{60} moiety, clearly shifts to a more positive potential, well outside the redox window of the C_{60} unit. The CV data for **2** are in accordance with data obtained for flavin derivatives recorded in the presence of a complementary diamidopyridine, as the wave for the reoxidation of $2_{red}H^-$ to **2** is virtually fully suppressed due to the prevention of the e–c–e process due to host–guest complexation.[11] Therefore, the data are consistent with a significant stabilisation of the 1_{rad}^- and 2_{rad}^- states upon the addition of **3** to the electrochemical cell, and a concomitant increase in the hydrogen bonding efficiency upon the electrochemical reduction of the flavin moiety of the complexes.

An interesting feature of the electrochemistry of derivatives **1** or **2** upon the addition of excess **3**, was the differing electrochemical data obtained for the first reduction wave of the C_{60} moiety. The first reduction wave was shifted to a more positive value for derivative **1** ($\sim$ +20 to +30 mV), whereas a negative shift for derivative **2** ($\sim$ −20 to −30 mV) was observed. The second reduction wave of the C_{60} moiety is largely unaffected by the addition of **3**. Negligible shifts in the reduction waves of derivative **4** were observed upon the addition of excess **3**, indicating that the host–guest binding between the flavin and diamidopyridine moieties has the ability to modulate the reduction potential of the first reduced state of the C_{60} moiety. This modulation demonstrates the ability of flavin to transduce supramolecular events into electronic changes in a remote substituent.

In conclusion, we have shown that the covalent attachment of a C_{60} unit to flavin derivatives influences the fluorescence and electrochemical properties of the latter. However, the C_{60} unit does not significantly impair the flavin's ability to undergo electrochemically-tuneable hydrogen bonding interactions with diamidopyridine derivative **3**. Indeed, significant redox-enhanced binding between the flavin moiety of **1** or **2** and **3** was observed upon electrochemical reduction of the flavin moieties to their radical anion states. We are currently exploiting these systems to create new molecular electronics components, and the results of these investigations will be reported in due course.

Notes and references

1 (a) E. Breinlinger, A. Niemz and V. M. Rotello, *J. Am. Chem. Soc.*, 1995, **117**, 5379; (b) A. Niemz and V. M. Rotello, *Acc. Chem. Res.*, 1999, **32**, 44.
2 (a) A. K. Boal and V. M. Rotello, *J. Am. Chem. Soc.*, 1999, **121**, 4914; (b) A. K. Boal and V. M. Rotello, *J. Am. Chem. Soc.*, 2000, **122**, 734; (c) A. K. Boal and V. M. Rotello, *J. Am. Chem. Soc.*, 2002, **124**, 5019.
3 For examples see: (a) Q. Xie, E. Perez-Cordero and L. Echegoyen, *J. Am. Chem. Soc.*, 1992, **114**, 3977; (b) C. Bruno, I. Doubitski, M. Marraccio, F. Paolucci, D. Paolucci and A. Zaopo, *J. Am. Chem. Soc.*, 2003, **125**, 15738.
4 For recent reviews see: (a) F. Diederich and M. Gómez-López, *Chem. Soc. Rev.*, 1999, **28**, 263; (b) D. M. Guldi and N. Martin, *J. Mater. Chem.*, 2002, **12**, 1978.
5 For recent reviews see: (a) T. Chuard and R. Deschenaux, *J. Mater. Chem.*, 2002, **12**, 1944; (b) G. Brusatin and R. Signorini, *J. Mater. Chem.*, 2002, **12**, 1964; (c) F. Wudl, *J. Mater. Chem.*, 2002, **12**, 1959.
6 T. D. Ros, G. Spalluto and M. Prato, *Croat. Chim. Acta*, 2001, **74**, 743.
7 A. Hirsch, *The Chemistry of the Fullerenes*, Thieme, New York, 1994.
8 M. Maggini, G. Scorrano and M. Prato, *J. Am. Chem. Soc.*, 1993, **115**, 9798.
9 All electrochemical experiments were performed using a CH Instruments 620A electrochemical workstation. The electrolyte solution (0.1 M) was prepared from recrystallised Bu_4NPF_6 and dry CH_2Cl_2. A three electrode configuration was used with a platinum disc working electrode, a platinum wire counter electrode and a silver wire pseudoreference electrode. Ferrocene was used as an internal reference with the ferrocene/ferrocenium couple adjusted to 0 V. The solutions were purged with nitrogen prior to recording the electrochemical data, and all measurements were recorded under a nitrogen atmosphere.
10 Although it is well established that it is possible to access up to four reduced states for C_{60} in CH_2Cl_2 (see ref. 3), derivatives **1** and **2** gave poorly defined redox waves for states $> 2^-$.
11 A. Niemz and V. M. Rotello, *J. Am. Chem. Soc.*, 1997, **119**, 6833.
12 (a) J. J. Hasford and C. J. Rizzo, *J. Am. Chem. Soc.*, 1998, **120**, 2251; (b) Y.-M. Legrand, M. Gray, G. Cooke and V. M. Rotello, *J. Am. Chem. Soc.*, 2003, **125**, 15789.
13 E. Breinlinger, A. Niemz and V. M. Rotello, *J. Am. Chem. Soc.*, 1995, **117**, 5379.
14 The thermodynamic data for these systems were calculated from binding constants determined by NMR titration experiments ($CDCl_3$) for complexes **3·5** (K_a = 540 ± 27 M^{-1}) and **3·6** (K_a = 385 ± 20 M^{-1}). The redox-based enhancement in recognition can be calculated using a thermodynamic cycle which is expressed mathematically using: $K_a(\text{red})/K_a(\text{ox}) = e^{(nF/RT)(E_{1/2}(\text{bound}) - E_{1/2}(\text{unbound}))}$. $K_a(\text{red})$ and $K_a(\text{ox})$ are the association constants of the reduced and oxidized forms, $E_{1/2}(\text{bound})$ and $E_{1/2}(\text{unbound})$ are the half-wave redox potentials of the receptor bound and unbound states, n is the number of electrons involved in the redox process and F is the Faraday constant.