

## Construction of a Stable Flavin–Gold Electrode displaying Very Fast Electron Transfer Kinetics

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The bis-phenylthiourea phenethylisoalloxazine (**6**) was synthesised and was attached to the surface of gold through its thiourea side-chains; cyclic voltammetric investigation of the redox properties of the system confirmed that the flavin was a stable adsorbed species and revealed that electron transfer between the conductor and the flavin was very fast.

Flavins serve as redox cofactors in a myriad of different biological transformations. As cofactors they are unique in their ability to participate in one- and two-electron transfer reactions. Attempts to employ flavins in the construction of electrodes have been reported over recent years but, in general, these systems show slow electron transfer rates or weak flavin–conductor interaction, or suffer from instability due to flavin desorption. In these studies the flavin was either adsorbed directly onto the conductor surface<sup>1</sup> or was covalently attached *via* a single linker arm.<sup>2</sup>

The X-ray crystal structure of the flavoprotein glutathione reductase has been determined to 3.0 Å resolution by Schulz

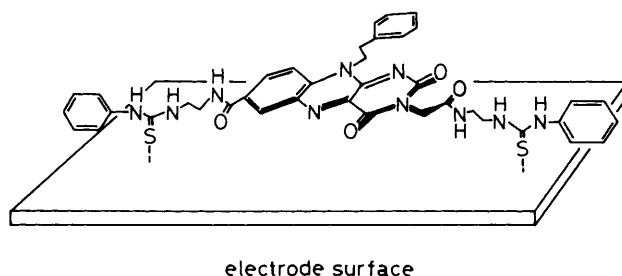
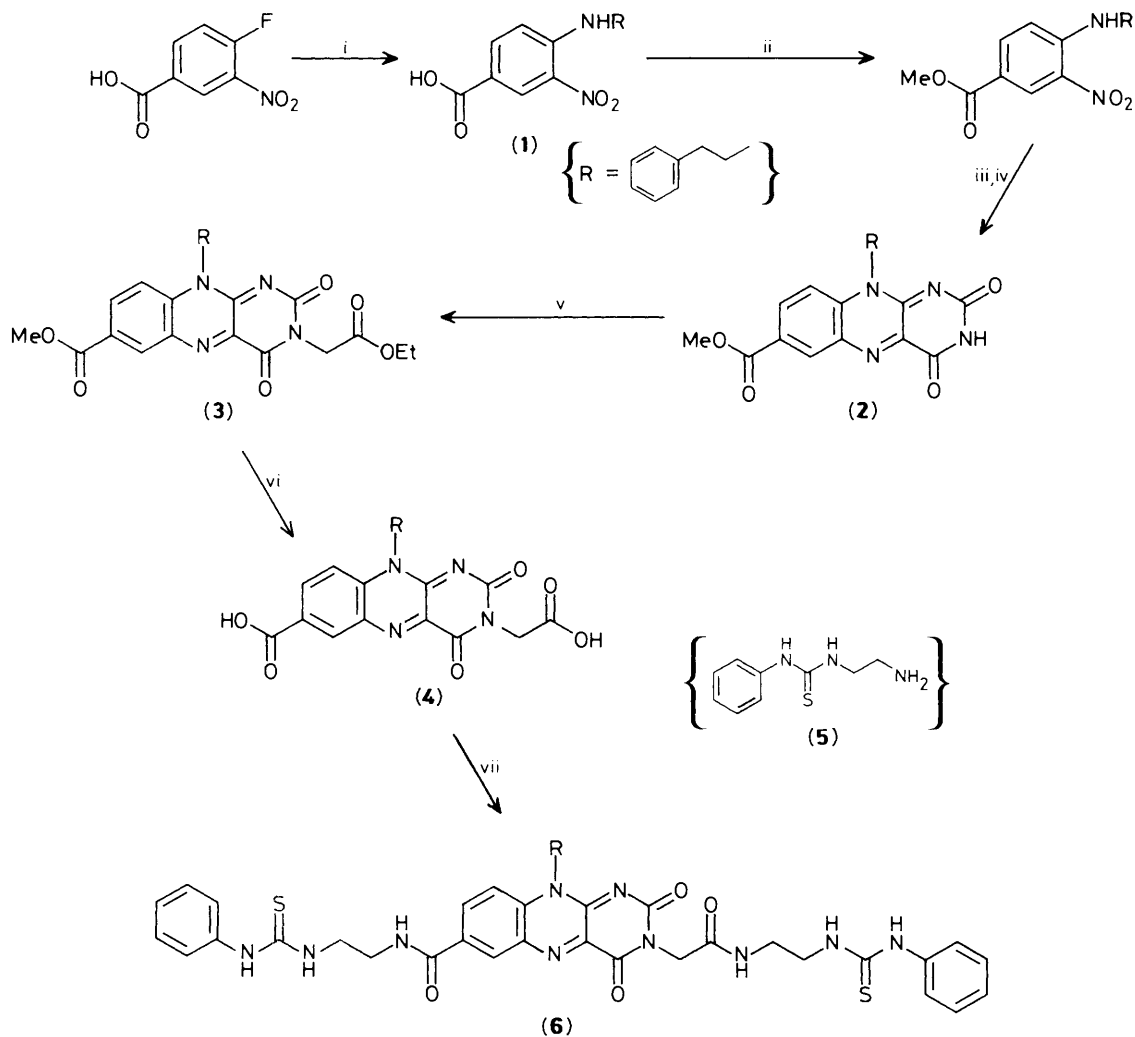
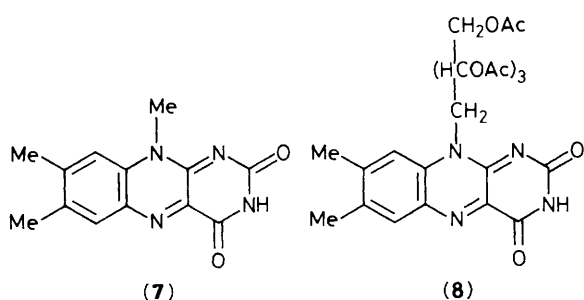


Figure 1. Orientation of the flavin with respect to the conductor.



**Scheme 1.** Reagents and conditions: i, phenethylamine, EtOH; ii,  $\text{SOCl}_2$ , MeOH; iii,  $\text{H}_2$ , Pd/C; iv, alloxan, HCl, EtOH; v,  $\text{K}_2\text{CO}_3$ , ethyl bromoacetate, DMF; vi, HCl,  $\text{H}_2\text{O}$ ; vii, BTBO, DMF, (5).

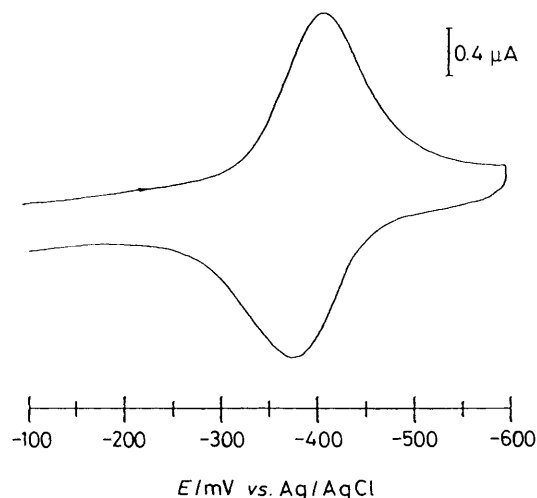


and co-workers<sup>3</sup> and the catalytic mechanism for the redox processes has been deduced. From this work it was evident that the electron donor, NADPH, and the oxidant, an active-site disulphide, are disposed on opposite faces of the flavin and that electrons must pass through the plane of the flavin during the reduction. In order to mimic this geometry in an electrode system, it was envisaged that the flavin should lie parallel to the surface of the conductor, Figure 1. Such an

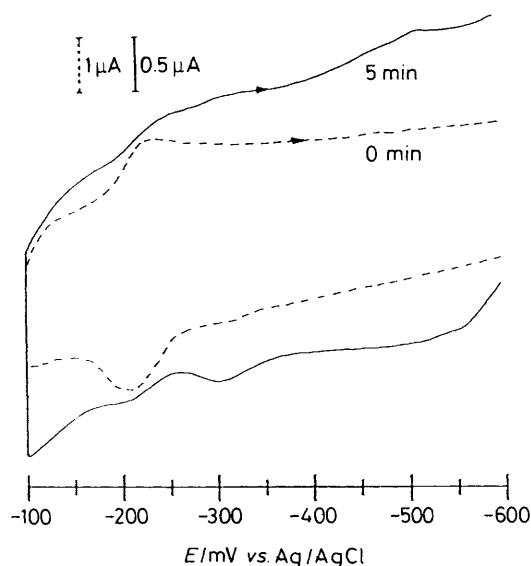
arrangement would require two points of attachment. Accordingly suitably bifunctionalised flavins were prepared for attachment to gold.

4-Fluoro-3-nitrobenzoic acid, prepared from 4-fluorobenzoic acid, was treated with phenethylamine to give the aromatic amine acid (1) which was further converted to the ester by treatment with thionyl chloride in methanol in 47% overall yield. Catalytic reduction of the nitro ester gave the diamine ester which was not isolated but immediately treated with alloxan under acidic conditions to give the isoalloxazine (2) in 64% yield.

The N-3 position of the isoalloxazine was alkylated with ethyl bromoacetate in the presence of potassium carbonate using a modification of the method of Kraus *et al.*<sup>4</sup> to give the diester (3) which was hydrolysed to the diacid (4). In model reactions attempts to couple the diacid to aliphatic amines using carbodiimide methodology or through the initial formation of the bis-acid chloride or bis-carbonic mixed anhydrides proved unsuccessful; however, the 1,1'-bis-[6-trifluoromethyl]-benzotriazolyl] oxalate (BTBO)-activated flavin diacid reacted smoothly.<sup>5</sup> Treatment of the BTBO-activated diacid with the aminoethyl thiourea (5), prepared from



**Figure 2.** Cyclic voltammogram of bis-phenylthiourea-phenethylisoxazine (**6**) adsorbed on a gold electrode, 0.1 M  $\text{KH}_2\text{PO}_4$ , 0.1 M KCl, pH 7.0, 50  $\text{mV s}^{-1}$ .



**Figure 3.** Cyclic voltammogram of lumiflavin (**7**) adsorbed on a gold electrode, 0.1 M  $\text{KH}_2\text{PO}_4$ , 0.1 M KCl, pH 7.0, 100  $\text{mV s}^{-1}$ .

1,2-diaminoethane and phenylisothiocyanate,<sup>6</sup> gave the desired bis-thiourea (**6**) in 62% yield, Scheme 1.†

The attachment of the bis-thiourea to gold was effected by dipping a cleaned electrode into a 1.3 mM solution of the flavin

† All compounds showed the expected spectral and analytical properties. For compound (**6**): m.p. 155 °C (decomp.);  $\lambda_{\text{max}}$  (DMF) 436 ( $\epsilon$  16 100  $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ ), 332 (12 900), and 280 nm (106 400);  $\nu_{\text{max}}$  1730w, 1670w, 1605m, and 1570w  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  (360 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ ) 3.15 [2H, t,  $J$  7.98 Hz,  $\text{N}(10)\text{CH}_2\text{CH}_2$ ], 3.45 (2H, br.t,  $\text{CH}_2$ ), 3.67 (2H, t,  $J$  2.76 Hz,  $\text{CH}_2$ ), 3.77 (2H, t,  $J$  4.94 Hz,  $\text{CH}_2$ ), 3.92 (2H, br.t,  $\text{CH}_2$ ), 4.76 (2H, s,  $\text{NCH}_2\text{CO}$ ), 4.88 [2H, t,  $J$  7.90 Hz,  $\text{N}(10)\text{CH}_2\text{CH}_2$ ], 7.10–7.35 (15H, ArH), 7.70 (1H, d,  $J$  9.22 Hz, 9-H), 8.30 (1H, dd,  $J$  2.05 and 9.06 Hz, 8-H), and 8.57 (1H, d,  $J$  2.08 Hz, 6-H);  $\delta_{\text{C}}$  (67.9 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$ ) 34.22 ( $\text{NCH}_2\text{CH}_2\text{Ph}$ ), 41.74 ( $\text{NCH}_2\text{CH}_2\text{Ph}$ ), 44.62 ( $\text{NCH}_2\text{CO}$ ), 45.42 ( $\text{CH}_2\text{NHCS}$ ), 45.77 ( $\text{CH}_2\text{NHCS}$ ), 48.07 ( $\text{CH}_2\text{NHCO}$ ), 117.66 (C-9), 126.03–139.27 (ArC), 150.86 (C-10a), 157.31 (C-2), 161.34 (C-4), 167.62 (C-3 $\beta$ ), 170.23 (C-7 $\alpha$ ), and 182.61 (C=S);  $m/z$  (fast-atom bombardment) 776 [33%, ( $M + H$ )<sup>+</sup>], 500 (25), 139 (27), 77 (100), and 63 (41).

in dimethylformamide (DMF). The excess of flavin was removed through extensive washing with DMF and then water. Lumiflavin (**7**) and tetra-acetylriboflavin (**8**) were treated similarly.

Cyclic voltammograms were recorded over a range of scan rates from 5  $\text{mV s}^{-1}$  to 1000  $\text{V s}^{-1}$  in 100 mM  $\text{KH}_2\text{PO}_4$  and 100 mM KCl in water at pH 7.0 under argon. While it was evident from the analysis of the voltammograms that the bifunctionalised flavin (**6**) was firmly attached to the surface of the gold electrode, the flavins which did not possess thiourea functionality and were readily desorbed from the surface of the electrode, Figures 2 and 3. For the flavin (**6**), a linear correlation of cathodic peak current with sweep rate was observed (correlation coefficient 0.997) which is indicative of an adsorbed electroactive species. Furthermore, the flavin electrode was stable to continuous potential cycling in the regions of water oxidation and reduction.

The formal heterogeneous rate constants for electron transfer were determined by the method of Daifuku *et al.*<sup>7</sup> from the linear portions of plots of peak potential vs.  $\ln$  sweep rate. The linear portion of the plots represent totally irreversible reactions. Through the use of an 'in-house' computer iteration program<sup>8</sup> the rate constants for the anodic and cathodic processes were determined. The values for the rate constants were  $5.6 \times 10^2$  and  $3.2 \times 10^3 \text{ s}^{-1}$  respectively, in phosphate buffer at pH 7.0. These results compare very favourably with previously reported rates. Ueyama *et al.*<sup>9</sup> calculated a cathodic rate constant of 1.22  $\text{s}^{-1}$  for a flavin Langmuir–Blodgett monolayer, whilst Daifuku<sup>7</sup> obtained values of  $1.33 \times 10^2$  and  $7.1 \times 10^1 \text{ s}^{-1}$  for complexes of osmium and ruthenium, respectively.

The results reported here indicate that the geometry of flavins attached to the surface of conductors is important in determining the electroactivity of the electrode systems and that parallel face attachment provides a mechanism for fast electron transfer. These systems and those containing three points of attachment for the flavin further offer the potential for detailed studies of the geometry, mechanism, and surface requirements of electron transfer at a fundamental level. Research in this area is in progress.

We thank Dr. D. Schiffrin, Wolfson Centre for Electrochemistry, for useful discussions and the S.E.R.C. for an IT studentship to T. R. G. E. and for financial support.

Received, 9th May 1989; Com. 9/01940E

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