# Photostimulated Imprinted Polymers: A Light-regulated Medium for Transport of Amino Acids

**Sharon Marx-Tibbon and Itamar Willner***

*Institute of Chemistry and Farkas Center for Light Induced Processes, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

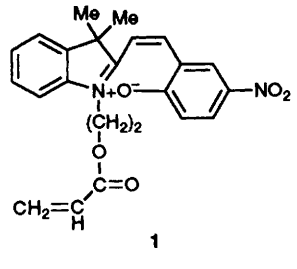
A tryptophan imprinted merocyanine copolymer exhibits transport properties towards the polymer-registered substrate; the photoisomerized spiropyran copolymer is non-permeable towards the substrate and the imprinted information is erased.

Imprinted polymers gained interest as a reaction medium for selective association,[1] separation[2] and catalytic transformations[3] of low molecular mass molecules. The principle of polymer imprinting consists of selecting the monomers by their ability to form non-covalent interactions (electrostatic, hydrogen bonds, hydrophobic, *etc.*) with a template molecule.[4] During the polymerization, the template molecule imprints a cavity in the polymer backbone, reflecting the non-covalent interactions of the host molecule. Extraction of the substrate after polymerization results in imprinted cavities in the polymer that are complementary in size, geometry and orientation to the substrate. Here we wish to report on the preparation of a light-sensitive imprinted polymer for the transport of tryptophan. We demonstrate that the registered information in the form of the imprint towards tryptophan can be switched off and triggered on by light.

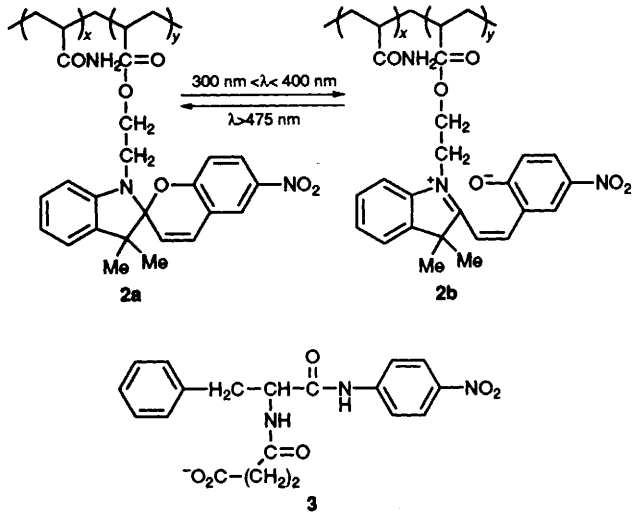
Spiropyrans undergo photoisomerization to the zwitterionic merocyanine isomer by irradiation with UV light.[5] The merocyanine form is capable of interacting electrostatically with the zwitterionic amino acid tryptophan. Hence, incorporation of a merocyanine monomer such as **1** in the polymerization mixture will provide a binding site for tryptophan.[6] A mixture consisting of acrylamide (5.2 mmol), *N,N'*-methylenebis(acrylamide) (0.4 mmol), acrylic acid (0.4 mmol), methyl methacrylate (0.5 mmol), ethylene glycol dimethacrylate (0.12 mmol) and **1**[7] (0.005 mmol) was polymerized in the presence of tryptophan (0.05 mmol).†

The affinity of the polymer towards the imprinted molecule, tryptophan, was examined by means of flow-dialysis.[8] Two chambers of the flow-dialysis cell were separated by the polymer membrane. The upper chamber contained a solution of 0.01 mol $dm^{-3}$ tryptophan, while water flowed through the lower chamber (flow rate = 30 ml $h^{-1}$). Fractions of the eluted solution were collected at time intervals and analysed fluorimetrically. The polymer membrane separating the two chambers was extensively washed prior to any dialysis experiment to ensure the absence of any entrapped tryptophan. The polymer membrane positioned in the cell, was irradiated by UV light (λ = 330–370 nm) to produce the coloured merocyanine membrane, **2b**, and with regular visible light to generate the spiropyran isomeric polymer, **2a**.

Fig. 1 shows the flow-dialysis profiles of tryptophan across the photoisomerizable polymer membranes, **2a** and **2b**. Upon illumination of the imprinted polymer with UV light, the polymer exists in state **2b**. Tryptophan permeates through the membrane, Fig. 1 curve (*a*), and a concentration of $1 \times 10^{-4}$ mol $dm^{-3}$ of the substrate is eluted from the lower chamber. Illumination of the imprinted polymer with visible light results in the polymer in state **2a**. Positioning of this membrane in the flow-dialysis system results in the elution profile for tryptophan shown in Fig. 1 (curve *b*). Thus, photoisomerization of the imprinted polymer to state **2a** blocks the permeation of the substrate across the membrane and a very low concentration, *ca.* $4 \times 10^{-6}$ mol $dm^{-3}$ is eluted from the lower chamber.‡ A control experiment was performed where a non-imprinted polymer of similar composition was applied as membrane in the states **2a** and **2b** in the dialysis experiment. Tryptophan is dialyzed across the non-imprinted polymers in states **2a** and **2b** similarly and very inefficiently. Less than $10^{-6}$ mol $dm^{-3}$ of tryptophan were detected in the eluted solutions in the two isomeric states **2a** and **2b**. Thus, we reveal that the crosslinked, non-imprinted polymer in states **2a** and **2b** exhibits poor permeabilities towards tryptophan. Imprint of the tryptophan pattern into the membrane structure during polymerization, however, generates a permeable copolymer. Presumably, electrostatic interactions between tryptophan and **1** generate




**1**



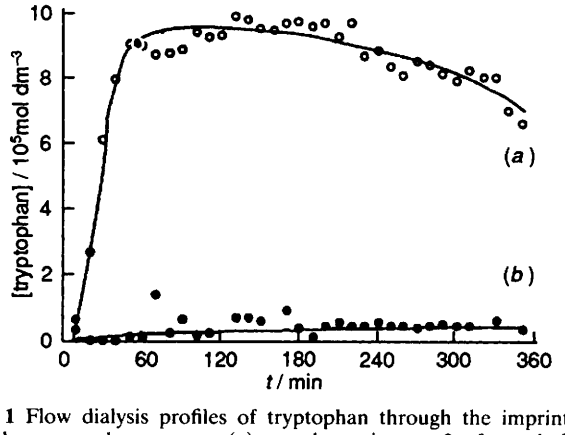




**Fig. 1** Flow dialysis profiles of tryptophan through the imprinted copolymer membrane, curve (*a*): membrane in state **2a**, formed after illumination with λ = 310–370 nm; curve (*b*): membrane in state **2b** formed by illumination with visible light

during polymerization the complementary cavities for the substrate. The structural information registered in the cavity is photochemically destroyed by isomerization of **2b** to **2a**. Thus, depletion of the zwitterionic site associated with the respective complementary cavities, and the structural isomerization of the photoactive components block the permeation of the substrate through the membrane.

In a further control experiment directed to reveal the selectivity of the tryptophan-imprinted copolymer towards permeation, we substituted tryptophan with *N*-(3-carboxypropionyl)-L-phenylalanine-*p*-nitroanilide, **3**, as the dialyzed substrate. This substrate is sterically larger than tryptophan and lacks the complementary electrostatic interactions with the tryptophan-imprinted cavities of **2b**.§ Fig. 2 shows the dialyses profiles of **3** across the tryptophan-imprinted copolymers in states **2a** and **2b**. The substrate was dialyzed through the membranes in states **2a** and **2b** at similar rates. Note, however, that permeation of **3** across these membranes is *ca.* 10-fold lower as compared to tryptophan permeation across **2b** (*ca.* $1.2 \times 10^{-5}$ mol $dm^{-3}$ of **3** is detected in the eluted solution). Thus, the polymer states **2a** and **2b** do not discriminate the permeation of **3** across the membrane. The lower rate in transport of **3** across the polymer can be attributed to inappropriate imprinted cavity configurations.

Thus, imprinting a polymer using a photoisomerizable monomer capable of non-covalent interactions with the print molecule provides a means to photoregulate the permeabilities of the polymer membrane. Imprinted photoisomerizable polymers can switch off the registered information and block the imprinted functions of the membrane. Application of such polymers as light-controlled release devices seems feasible. Utilization of photoisomerizable imprinted polymers for light-regulated catalysis and chromatography are currently under way in our laboratory.

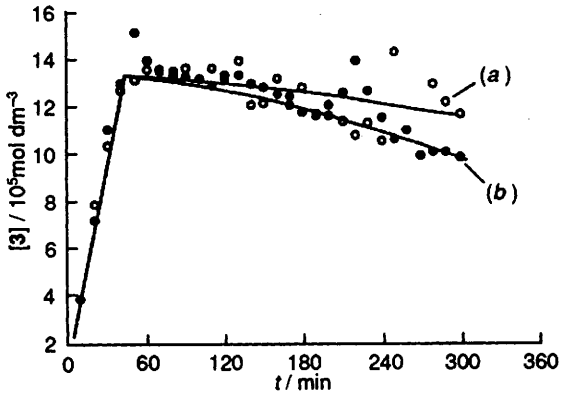



**Fig. 2** Flow dialysis of **3** through the tryptophan-imprinted membrane, curve (*a*): membrane in state **2b**, formed after illumination with λ = 310–370 nm; curve (*b*): membrane in state **2a** formed by illumination with visible light

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## Footnotes

† Polymerization was initiated by potassium peroxodisulfate and 3-(dimethylamino)propionitrile. Membranes were produced by polymerization of the ingredients between two glass plates separated by a 0.5 mm spacer. The membranes were washed and kept in an aqueous solution, 4 °C, for at least 12 h prior to their use.

‡ The polymer membranes **2a** or **2b** reveal poor mechanical strength. Attempts to photostimulate reversible ON–OFF switchable transport of tryptophan across the membranes failed as they were torn upon photoisomerization to **2b** or **2a**, respectively, in the dialysis assembly. Nonetheless, polymer **2a** could be reversibly photoisomerized to state **2b** and back to **2a**, outside the dialysis chamber, for at least 10 cycles. Under these conditions any copolymer in state **2a** revealed poor permeability towards the substrate while any copolymer in state **2b** exhibited high permeability for tryptophan, when mounted in the flow dialysis system.

§ Phenylalanine, which exhibits structural similarity to tryptophan, reveals permeability properties similar to those of the imprinted substrate. That is, it is permeable across tryptophan imprinted **2b** and non-permeable across **2a**.

## References

1 R. Arshady and K. Mosbach, *Makromol. Chem.*, 1981, **182**, 687; T. Rosatzin, L. I. Andersson, W. Simon and K. Mosbach, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1261.

2 B. Sellergen, M. Lepisto and K. Mosbach, *J. Am. Chem. Soc.*, 1988, **110**, 5853; O. Nörrlow, M. Glad and K. Mosbach, *J. Chromatog.*, 1984, **299**, 29; L. Fischer, R. Muller, B. Ekberg and K. Mosbach, *J. Am. Chem. Soc.*, 1991, **113**, 9358.

3 D. K. Robinson and K. Mosbach, *J. Chem. Soc., Chem. Commun.*, 1989, 969.

4 For a review see G. Wulff, in *Polymeric Reagents and Catalysts*, ed. W. T. Ford, ACS Symposium Series 308, ACS Washington DC 1986, USA.

5 R. Guglielmetti, in *Photochromism-Molecules and Systems*, Studies in Organic Chemistry 40, ed. H. Dürr and H. Bouas-Laurent, Elsevier, Amsterdam, 1990, p. 314.

6 J. Sunamoto, K. Iwamoto, Y. Mohri and T. Kominato, *J. Am. Chem. Soc.*, 1982, **104**, 5502.

7 This monomer was synthesized according to A. Hassner and A. Vazken, *Tetrahedron Lett.*, 1987, **46**, 4475.

8 I. Willner, S. Rubin, R. Shatzmiller and T. Zor, *J. Am. Chem. Soc.*, 1993, **115**, 8690; I. Willner and S. Rubin, *Reactive Polymers*, 1993, **21**, 177; S. P. Colowck and F. C. J. Womak, *J. Biol. Chem.*, 1974, **244**, 744.