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Direct Evidence for the Importance of Hydrophobic Mismatch for Cell Membrane Recognition

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Abstract In this *Letter*, we describe the synthesis of amphiphilic oligo(*p*-phenylene)s from 31 to 44 Å length and delineate the interaction of these rigid-rod molecules with lipid bilayers using fluorescence quenching methods. The results demonstrate high importance of hydrophobic mismatch for selective cell membrane recognition by rigid-rod molecules.

The interaction of hydrophobic guests with host lipid bilayers is governed by hydrophobic matching. Several theoretical models have implied that the thickness of the host membrane (d_H) can adjust to the length of the hydrophobic guest (d_G) by stretching ($d_G > d_H$) and squashing or tilting ($d_G < d_H$) of the flexible lipid hydrocarbon chains (Fig. 1).[1] Because of the energy needed to deform lipid bilayers, the binding of guests to matching host bilayers ($d_G = d_H$) should occur selectively with respect to mismatched membranes ($d_G \neq d_H$). However, although hydrophobic matching is considered to be crucial for biological activity within lipid bilayers,[2-5] there is surprisingly little experimental evidence for its importance for cell membrane recognition (i.e., preferential guest binding to matched compared to mismatched host membranes).[6-9] Rigid-rod fluorophores are unique synthetic models to obtain such information because changes in guest length can be excluded, and differentiation between specific, transmembrane binding due to hydrophobic matching and nonspecific binding to the surface or in-between the leaflets of the bilayer is possible using fluorescence quenching methods.[7,9] Recent studies with rigid-rod fluorophores of 17 to 34 Å length have revealed reduced ion transport activity with increasing ($d_G < d_H$) - mismatch,[8] but no indications of cell membrane recognition have been found because of nonspecific binding.[7,9] Here we demonstrate high significance of ($d_G > d_H$) - mismatch for cell membrane recognition using newly devised amphiphilic rigid-rod fluorophores of 31 to 44 Å length as guests for biomimetic EYPC host bilayers.[10]

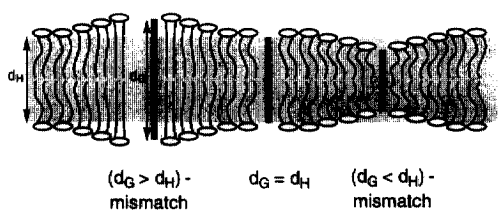

Figure 1

The oligo(*p*-phenylene)s **1** - **4** were designed with an ionic iminodiacetate (IDA) side chain at one terminus of the rigid-rod scaffold to assure sufficient and comparable solubility of the homologous fluorophores of up to

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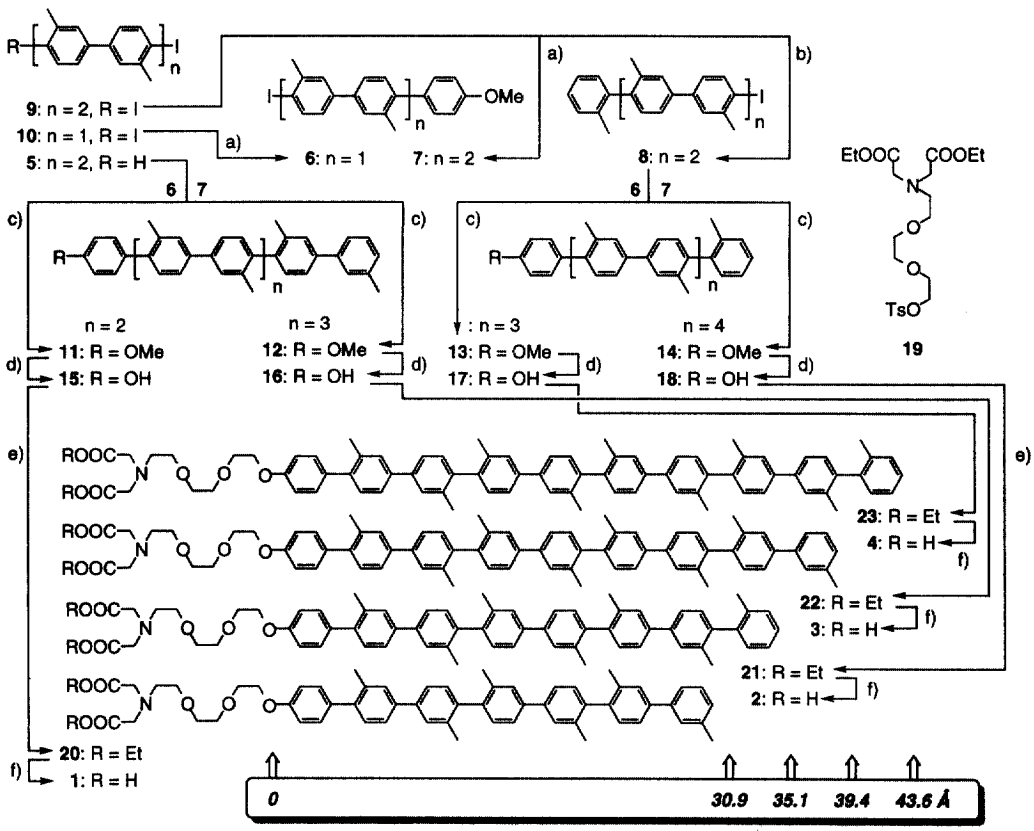
44 Å length, to prevent nonspecific interactions with the host bilayer, and for optional binding of ligands to the ion-chelating IDA located at the host membrane surface in the case of specific transmembrane binding of the hydrophobic guest (Scheme 1).[11] The monoiodo building blocks **5** - **8** for the synthesis of **1** - **4** were either previously reported (**5**)[12] or accessible by Suzuki coupling[13] of the known oligotoluenes **9** and **10** with the corresponding aryl boronic acids. Cross-coupling of the haloarenes **5** - **8** using the very recently introduced "Suzuki-Giroux" one-pot methodology[14] afforded the rigid-rod homologs **11** - **14** in excellent 40 - 48% yield. The IDA side chains were attached as before by aryl methyl ether cleavage of the anisoles **11** - **14** followed by Williamson ether synthesis with the resulting phenols **15** - **18** and tosylate **19** to give the ethyl esters **20** - **23**.[15] Reesterification of the rigid-rod amphiphiles **1** - **4** obtained from hydrolysis of **20** - **23** was required for satisfactory spectroscopic characterization as reported before for similar cases.[15,16]

Binding of the rigid-rod guests **1** - **4** with 31 to 44 Å length to biomimetic EYPC host bilayers of 43 Å

Scheme 1



a) 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, 8 h, 80 - 100°, 42 - 43% (conversion yield: 61 - 64%); b) 2-methylphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, 8 h, 80 - 100°, 47% (conversion yield: 57%); c) 1. **6** / **7**, bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMF, 2 h, 80°, 2. **5** / **8**, $PdCl_2$(dppf), 2 M aq. Na_2CO_3, 8 h, 80°, 40 - 48%; d) BBr_3, CH_2Cl_2, 14 h, -78° → rt, 82 - 91%; e) **19**, Cs_2CO_3, DMF, 14 h, 55°, 59 - 71%; f) aq. NaOH, THF / MeOH, 1 h, rt, quant.

overall thickness[17,18] was investigated using lipids that carry a fluorescence quencher near the beginning (5-DOXYL-PC) or the end of the hydrophobic tail (12-DOXYL-PC).[9,10] Because of the short effective distance of quenchers,[19] these two labels can be used to distinguish between specific, transmembrane binding (quenching by 5-DOXYL-PC = 12-DOXYL-PC) and nonspecific binding to the surface (quenching by 5-DOXYL-PC > 12-DOXYL-PC) or in-between the bilayer leaflets (quenching by 5-DOXYL-PC < 12-DOXYL-PC).[7,9] Figure 2A shows the comparison of the intensity of the emission maxima of 2.5 μM rigid-rod fluorophores **1** - **4** between 364 and 370 nm in the presence of 250 μM EYPC-SUVs with (I) and without 2% DOXYL-PC (I_0) after 1 h incubation at room temperature (excitation maxima of **1** - **4** were between 283 and 295 nm). It is seen that at low lipid / sample ratios both septi(*p*-phenylene) **1** (31 Å) and octi(*p*-phenylene) **2** (35 Å) undergo transmembrane binding to EYPC bilayers, while nonamer **3** (39 Å) and decamer **4** (44 Å) do not exhibit significant specific quenching. Reduction of the fluorophore concentrations with constant EYPC-SUV concentrations revealed saturation at lower lipid / sample ratios for **1** (31 Å) than for **2** (35 Å, Fig. 2, ■, ●). Although likely, the reduced quenching observed for **3** (39 Å) at saturation is not acceptable as unambigous proof for reduced binding (Fig. 2, ◆). However, clear evidence for insignificant binding due to increased ($d_G > d_H$) - mismatch was found for **4** (44 Å), because weak quenching is seen together with unsaturation up to a lipid / sample ratio of 10,000 (Fig. 2, ▲). The above experiments were repeated with constant sample concentrations (125 nM) and varied lipid concentrations to exclude the possibility that changes in solubility or micelle formation of the amphiphiles **2** - **4** account for the observed effects of ($d_G > d_H$) - mismatch (Fig. 2b, ❑, Δ, ◊). However, identical trends were observed in all experiments, implying that the binding of hydrophobic guest to host bilayers is negligible if the length of the guest exceeds the *overall* thickness of the host bilayer. The perhaps counter-intuitive finding that ($d_G > d_H$) - mismatched decamer **4** preferably remains dissolved in buffer instead of incorporating into the hydrophobic bilayer or precipitating might be better understood by considering the low concentrations under investigation as well as the possiblility of micellization of the rigid-rod amphiphile.

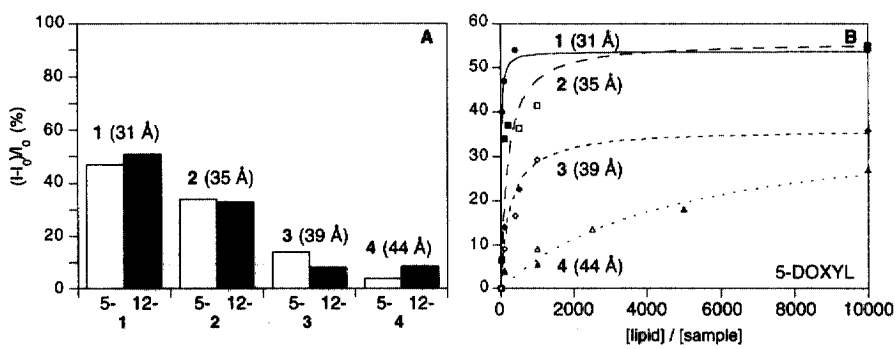


Figure 2

In summary, we have synthesized four homologous rigid-rod fluorophores of 31, 35, 39, and 44 Å length to delineate the importance of ($d_G > d_H$) - mismatch for cell membrane recognition. The results imply that the binding of hydrophobic guest to host bilayers depends on hydrophobic matching to an extent that was not necessarily expected from theoretical models.[1] With the results of this study, it is now predictable that hydrophobic matching of rigid-rod guests presumably results in a) selective recognition of the corresponding

host membrane from thinner membranes ($(d_G > d_H)$ - mismatch) and b) nonspecific binding to thicker membranes ($(d_G < d_H)$ - mismatch).[7-9] The remarkable selectivity seen for ($d_G > d_H$) - mismatch is of particular practical importance, because it implies that antimicrobial agents with a length that hydrophobically match the mycobacterial barrier[20] would not interact with mammalian cell membranes, i.e., low toxicity of such potential antimycobacterials.

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10. Abbreviations: 5-DOXYL-PC: 1-palmitoyl-2-stearoyl(5-DOXYL)-*sn*-glycero-3-phosphocholine; 12-DOXYL-PC: 1-palmitoyl-2-stearoyl(12-DOXYL)-*sn*-glycero-3-phosphocholine; EYPC: egg yolk phosphatidylcholine; IDA: iminodiacetate; SUV: small unilamellar vesicle.
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16. All products except **1** - **4** (see text and ref. 15) gave satisfactory spectroscopic data. For example: **20:** FAB-HRMS: calc. for $C_{62}H_{68}NO_7$: 938.49598. Found: 938.49957. **21:** FAB-HRMS: calc. for $C_{69}H_{74}NO_7$: 1028.54406. Found: 1028.54651. **22:** FAB-HRMS: calc. for $C_{76}H_{80}NO_7$: 1118.59112. Found: 1118.59351. **23:** FAB-HRMS: calc. for $C_{83}H_{86}NO_7$: 1208.64824. Found: 1208.64038.
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