Communications

distributed by the Environmental and Molecular Sciences Laboratory, Richland, WA, USA, and funded by the U.S. Department of Energy.

[13] To rationalize the different steric requirements for the horizontally and vertically arranged borylene ligand a "ϕ-ψ" search was performed. In this the dihedral angles H-C-Si-N, C-Si-N-B, and C-Cp$_m$-V-B were changed in 5° steps over a range of 120° for the former two and 72° for the latter one, and the distances between terminal atoms of different groups, that is Cp, Me, and CO were calculated. For geometries with the smallest interatomic distances the search was repeated in 2° steps over ranges of ±60° and ±36°. The following distances [Å] were determined; vertical: Cp−Me 1.633 (smallest distance), 2.412 (average distance), CO−Me 1.652 (smallest distance), 2.308 (average distance); horizontal: Cp−Me 1.959 (smallest distance), 2.887 (average distance), CO−Me 1.214 (smallest distance), 1.926 (average distance). While Cp−Me distances are comparable and even somewhat larger for the horizontal orientation, the very small CO−Me distances for the latter are evident. Although those results are of qualitative nature, they clearly provide evidence that the horizontal arrangement is sterically less favored.

[14] C. K. Fair, MolEN, An Interactive Intelligent System for Crystal Structure Analysis, Enraf-Nonius, Delft, The Netherlands, 1990.

[15] G. M. Sheldrick, Program Package SHELXTL, Version 5.1. Bruker Analytical X-ray Systems, Inc., Madison, Wisconsin, USA, 1998.
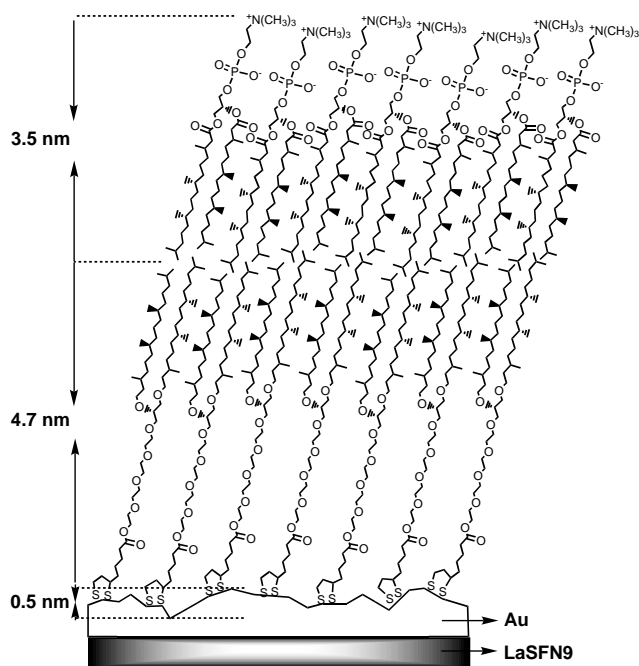
Figure 1. Schematic representation of the arrangement of the DPTL molecules to form the tBLMs. Molecular dimensions are in proportion to the ultrasmooth gold surface to guarantee the perfect alignment of the phytanyl chains in the formation of a dense mono- and bilayer. The tetraethylene glycol chains are assumed to be stretched. LaSFN9 = high-refractive-index glass.

Model Systems for Membranes

Archaea Analogue Thiolipids for Tethered Bilayer Lipid Membranes on Ultrasmooth Gold Surfaces**

Stefan M. Schiller,* Renate Naumann, Katherine Lovejoy, Horst Kunz, and Wolfgang Knoll

Tethered bilayer lipid membranes (tBLMs) mimick the biological cytoplasmic membrane and are promising tools for basic research and biosensor applications. They comprise

[*] Dipl.-Chem. S. M. Schiller, Dr. R. Naumann, Prof. Dr. W. Knoll
Max-Planck-Institut für Polymerforschung
Ackermannweg 10, 55128 Mainz (Germany)
Fax: (+49) 6131-379-100
E-mail: schiller@mpip-mainz.mpg.de

K. Lovejoy
Department of Chemistry, Northwestern University
Evanston, IL 60208-3113 (USA)

Dipl.-Chem. S. M. Schiller, Prof. Dr. H. Kunz
Institut für Organische Chemie, Universität Mainz
Duesbergweg 10–24, 55128 Mainz (Germany)

a lipid group linked to a metal surface, preferably gold, through a thiol-modified tether as spacer unit between the lipid and the gold substrate. The tether/spacer molecules should decouple the membrane from the surface and thus provide a hydrophilic layer (between the membrane and the substrate) with the functional properties of the cytosol/cytoskeleton.[1] They should establish a water-containing submembrane space, which reduces the hydrophobic influence of the metal surface,[2] thus enabling the functional incorporation of membrane proteins.[3]

Moreover, the combination of such ultrathin tBLMs with the robustness of a gold support in a planar configuration allows the application of surface-sensitive techniques, including surface plasmon resonance spectroscopy (SPR), measurements with a quartz-crystal microbalance (QCM), surface-enhanced IR, and electrochemical techniques such as electrochemical impedance spectroscopy (EIS). The metal surface also allows the investigation of surface molecules under a defined electric field, thus extending the scope of experimental conditions significant to the study of field-sensitive processes, for example, ion transport through receptors, channels, and carriers such as valinomycin.

The tBLMs reported so far are supplemented with polar peptide,[4] oligomer,[5] or polymer[6–8] spacers. Poly- or oligomeric tether molecules based on poly- or oligo(ethylene glycol) modified with dialkyl lipids tails have been employed in many cases. However, the capacitance and impedance of these tBLMs were found in the ranges 0.5–0.7 µF cm^{-2} and 0.01–0.1 MΩ cm^2, respectively,[5,6,9,10] insufficient to match the electrical properties of biological membranes. The model

surface binding residue | **polar tether group** | **hydrophobic ether linked isoprenoid tails**
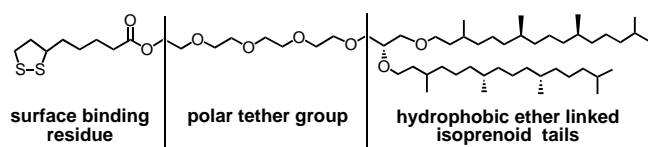
system that best reflects the electrical properties of the biomembrane is the bilayer lipid membrane (BLM), which shows capacitance values of around 0.5 µF cm⁻² and impedance values of ≥ 10 MΩ cm^2.[11,12] tBLMs with good electrical insulation properties were presented by Cornell et al.,[13] who used a multicomponent system consisting of a monophytanoyl spacer lipid together with a transmembrane lipid.

In contrast, the tBLM presented herein is based on a single molecule, the novel archaea analogue 2,3-di-*O*-phytanyl-*sn*-glycerol-1-tetraethylene glycol-D,L-α-lipoic acid ester lipid (DPTL). Phytanyl chains were chosen as hydrophobic tails instead of alkyl chains because of their low phasetransition temperature and their influence on the density and stability of biological membranes.[14] The phase-transition temperature of 2,3-di-*O*-phytanyl-*sn*-glycerol-1-tetraethylene glycol was determined to be $< -80\,°$C by differential scanning calorimetry (DSC). In contrast to the system of Cornell et al.[13] which bears one phytanoyl chain, our system has two phytanyl chains bound to the spacer through a chiral glycerol unit since it is known that a single hydrophobic chain does not guarantee a stable insertion of molecules into a lipid membrane.[15] Furthermore, the 2,3-di-*O*-phytanyl-*sn*-glycerol unit contains only ether linkages to prevent hydrolytic cleaving. This moiety is known to form stable biomembranes under the extreme living conditions (e.g. high temperatures) of extremophiles or archaea.[16] The importance of the ether linkage and the absence of esters and polar phosphatidyl groups to form highly impermeable membranes was experimentally demonstrated by Mathai et al.[17]

According to FTIR spectroscopic studies, the tetraeth−ylene glycol spacer can be considered as an elongated hydrophilic chain (Figure 1), which provides the maximum decoupling distance of the lipid membrane from the substrate. Other oligoethylene glycol (OEG) moieties such as penta- to octa(ethylene glycol) moieties adopt a 7/2 helical structure; the octa(ethylene glycol) appears to have a slightly distorted helical conformation.[18] The synthesis of DPTL is illustrated in Scheme 1.

The influence of structure and morphology of the gold substrate on the preparation of self-assembled monolayers (SAMs) is often disregarded. Herein we introduce

another key feature, the application of the ultrasmooth surface of template-stripped gold (TSG),[19] to form tBLMs with good insulating properties. Smooth metal surfaces such as that of mercury have been shown to be effective for the formation of tBLMs.[20]

The roughness of TSG was shown to be 0.5 nm over areas of µm^2 by AFM, one order of magnitude smaller than the molecular dimension of the DPTL molecule itself (Figure 1). This ensures the perfect supramolecular alignment of the functional units, resulting in a perfect arrangement of the monolayer. For comparison, the roughness of polycrystalline gold often used for the preparation of SAMs can be as high as 5–10 nm. Au (111), obtained by annealing the gold surfaces, has a roughness of 5 nm over an area of several µm^2 (measured by AFM, not shown). Rough surfaces seem to be a cause of the low impedance values observed in many model membranes.

High resistive tBLMs have been prepared from a SAM of DPTL on the TSG surface. The fusion of liposomes prepared from diphytanoylphosphatidylcholine (DPhyPC) gave rise to the formation of well-defined lipid bilayers as shown by SPR

Table 1: Data obtained by simulations from EIS (capacitance and resistance, Figure 2) and SPR (thickness) measurements. Molecule sizes obtained by modeling calculations with CS Chem 3D Pro are added for comparison.

	C_{II} (exp.) [µF cm⁻²]	R_{II} (exp.) [MΩ cm^2]	d (exp.) [nm]	d (calcd) [nm]
DPTL, monolayer before vesicle spreading	0.55	1.75	4.7	4.7
lipid bilayer after vesicle spreading	0.49	4.35	8.5	8.2
lipid bilayer in KCl (0.1 M) + valinomycin	0.64	0.0022		
lipid bilayer in KCl (0.1 M) + valinomycin after washing with NaCl (0.1 M)	0.61	0.31		
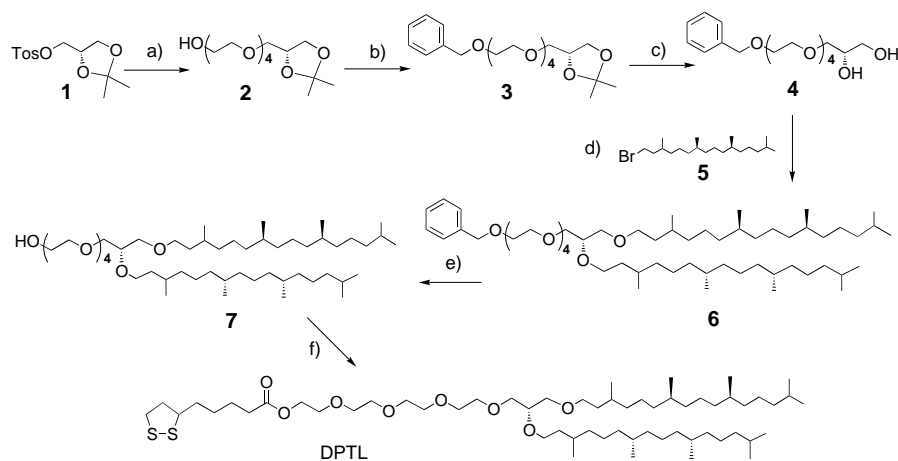
Scheme 1. Synthesis of DPTL: Acetal **1** was synthesized from D-mannitol according to reference [19]. a) Tetra(ethylene glycol) (20 equiv), NaH (2.5 equiv), dioxane, DMAP (cat.), Ar, 45–75 °C, 3 d; b) NaH (2 equiv), BnBr (2.5 equiv), TBAI (cat.), DMF, Ar, 0→20 °C, 2 d, 35 % (over steps a) and b)); c) H₂O/THF, Dowex 50 WX8, 50 °C, 18 h, 89 %; d) NaH (2.1 equiv), **5** (4 equiv, synthesized from phytol according to reference [20]), DMF, Ar, room temperature, 4 d, 49 %; e) THF, MeOH, H₂, Pd/C (0.3 equiv), room temperature, 30 min, quant.; f) lipoic acid (5 equiv), EDC (6.6 equiv), DMAP (cat.), CH₂Cl₂, Ar, 1 d, 60 %. Bn = benzyl, TBAI = tetrabutylammonium iodide, DMF = *N,N*-dimethylformamide, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, DMAP = 4-dimethylaminopyridine.

and EIS measurements. We have found only recently that SPR and EIS can be applied simultaneously not only to polycrystalline gold[21] but also to the TSG surface, provided that an optical glue is used for the preparation.[21] Hence this powerful combination of techniques was used to investigate the formation of tBLMs based on the DPTL molecule. Layer thicknesses were calculated from SPR measurements on the basis of a three-layer system of glass, gold, and the lipid layer. For the latter, a refractive index of $n = 1.5$ is assumed and the glue was assumed to have the same refractive index as glass. Results are given in Table 1. The thickness values are in agreement with the dimensions of the molecules, which were modeled with CS Chem 3D Pro (CambridgeSoft Corporation, Version 4.0). The configuration represented in Figure 1 shows a slight tilt angle of the molecular axis and the extended conformation of the OEG spacer. The increase in the thickness of the layer by fusion of the liposomes was monitored in real time by means of SPR spectroscopy and clearly showed saturation within 60 min, which indicates the unrolling of vesicles on a hydrophobic surface to form a lipid bilayer.

The impedance spectra are shown in Figure 2. From these spectra, the capacitance and the resistance of the lipid mono- and bilayer were calculated by fitting the data to the equivalent circuit.[22, 24, 25] The circuit consists of a RC mesh representing the lipid film, the resistance of the electrolyte solution, and the capacitance of the diffuse double layer adjacent to the gold. The results are given in Table 1. Data measured for the tBLMs varied from 0.45 to 0.8 μF cm^{-2} for the capacitance and from 2 to 12 MΩ cm^2 for the resistance. They thus compare quite well with those of the BLMs.

Also shown in Figure 2 is the effect of valinomycin on the resistance of the tBLM in the presence of potassium ions. Valinomycin is a potassium ionophore antibiotic, produced by *Streptomyces fulvissimus*,[26] which specifically includes potassium ions in its hydrophilic interior, while the hydrophobic exterior enables transmembrane transport of the potassium ions. This gives rise to the so-called inflection point in the EIS spectrum, at which point the resistance drops to the kΩ range (Table 1) and the capacitance of the diffuse double layer evolves to approximately 5 μF cm^{-2}, in agreement with values reported by other authors.[22, 24, 25]

It is concluded from these experiments that tBLMs can be prepared on SAMs of DPTL simply by vesicle fusion; the molecules are arranged almost perpendicular to the surface, particularly on ultrasmooth gold surfaces. The obtained tBLMs are stable over days and have electrical properties that resemble those of the biological membrane. The tetraethylene glycol spacer allows the formation of a diffuse double layer in the aqueous space between the lipid layer and the substrate, thus mimicking the cytoskeleton.[22, 24] The tBLM presented herein can thus be seen as model system for biological membranes.

Experimental Section

Au(111) surfaces and TSG: 60-nm gold films were deposited by electrothermal evaporation (0.01–0.05 nm s^{-1}, 2×10^{-6} mbar) on freshly cleaved mica sheets. The gold surface was annealed by heating
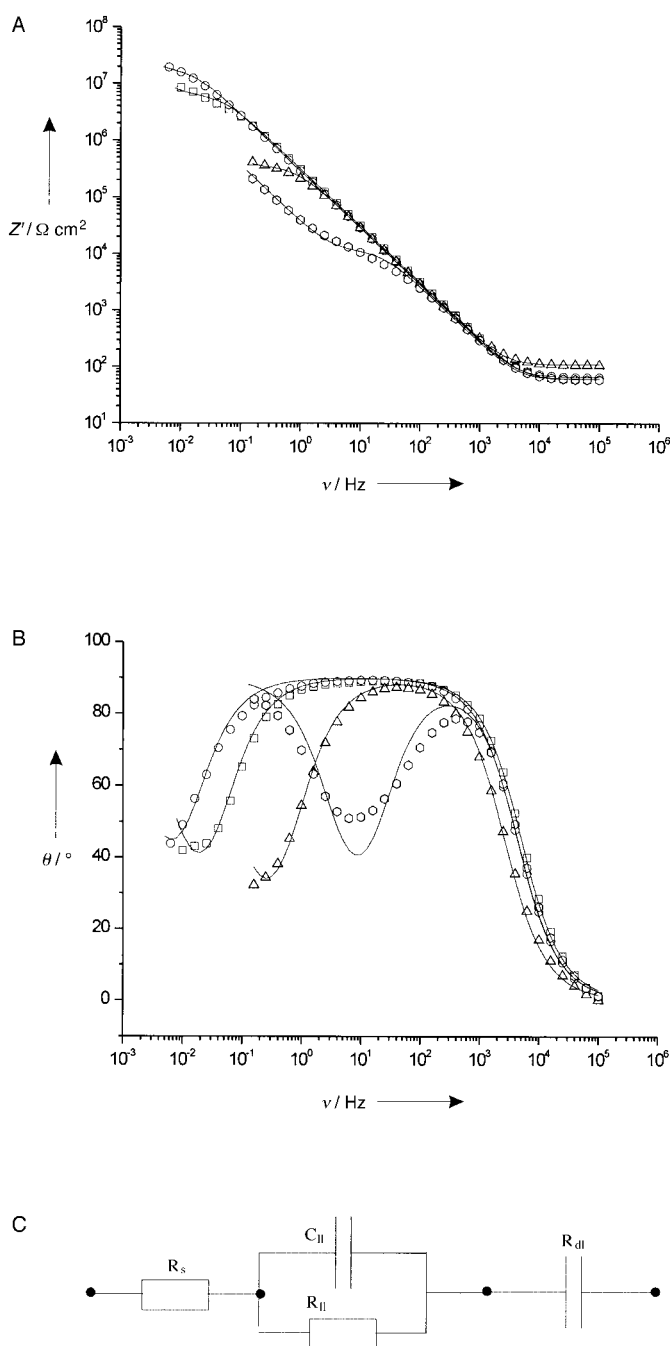

Figure 2. EIS analyses of TSG films coated with DPTL monolayers before (□) and after (○) fusion with DPhyPC liposomes in KCl solution (0.1 mol L^{-1}), the latter after adding valinomycin (0.1 μM) (hexagons), and after washing with NaCl (0.1 mol L^{-1}) (△). The solid lines represent fitted data. A) Z' (real part of the impedance) versus ν (frequency); B) θ (phase shift or phase angle) versus ν (frequency); C) equivalent circuit used for fitting purposes.

the mica sheets at 650 °C for 45 s to form Au(111). The gold surface was then glued with EPO-TEK 377 (Polytec GmbH, Waldbronn, Germany) to high-refractive-index ($n = 1.7$) glass slides and heated at 150 °C for 60 min. After cooling, these slides were immersed in THF for 30 s to allow the mica sheets to be separated from the gold film, which were then thoroughly rinsed with ethanol.

Formation of SAMs and vesicle fusion: TSG slides were placed for 24 h in an ethanolic solution of DPTL (0.2 mg mL^{-1}), rinsed in

pure ethanol, and dried under a stream of nitrogen. The prepared slides were then mounted in a measuring cell described earlier[23] for simultaneous SPR and EIS experiments. The thickness of the SAMs were measured in a solution of KCl (0.1 mol L^{-1}) before and after the addition of DPhyPC vesicles (DPhyPC supplied from Avanti Polar Lipids, Inc., Alabaster, Al, USA) freshly prepared by extrusion through a 50-nm polycarbonate filter. Fusion of vesicles was carried out at 30 °C at a final concentration of 0.02 mg mL^{-1}. EIS spectra were taken before and after the addition of valinomycin (final concentration = 4×10^{-6} mol L^{-1}). Surface plasmons were excited by a 632.5-nm HeNe laser. EIS measurements were conducted at a bias potential of 0 V vs. Ag/AgCl, saturated NaCl reference electrode and a platinum counter electrode.

[1] E. Sackmann, *Science* **1996**, *271*, 43.
[2] W. J. Miller, N. L. Abbott, *Langmuir* **1997**, *13*, 7106.
[3] R. Naumann, E. K. Schmidt, A. Jonczyk, K. Fendler, B. Kadenbach, T. Liebermann, A. Offenhäusser, W. Knoll, *Biosens. Bioelectron.* **1999**, *14*, 651.
[4] E. K. Schmidt, T. Liebermann, M. Kreiter, A. Jonczyk, R. Naumann, A. Offenhäusser, E. Neumann, A. Kukol, A. Maelicke, W. Knoll, *Biosens. Bioelectron.* **1998**, *13*, 585.
[5] H. Lang, C. Duschl, H. Vogel, *Langmuir* **1994**, *10*, 197.
[6] M. L. Wagner, L. K. Tamm, *Biophys. J.* **2000**, *79*, 1400.
[7] C. Erdelen, L. Häussling, R. Naumann, H. Ringsdorf, H. Wolf, J. Yang, M. Liley, J. Spinke, W. Knoll, *Langmuir* **1994**, *10*, 1246.
[8] J. Spinke, J. Yang, H. Wolf, M. Liley, H. Ringsdorf, W. Knoll, *Biophys. J.* **1992**, *63*, 1667.
[9] S. Heyse, O. P. Ernst, Z. Dienes, K. P. Hofmann, H. Vogel, *Biochemistry* **1998**, *37*, 507.
[10] L. M. Williams, S. D. Evans, T. M. Flynn, A. Marsh, P. F. Knowles, R. J. Bushby, N. Boden, *Langmuir* **1997**, *13*, 751.
[11] G. Wiegand, N. Arribas-Layton, H. Hillebrand, E. Sackmann, P. Wagner, *J. Phys. Chem. B* **2002**, *106*, 4245.
[12] a) R. Guidelli, G. Aloisi, L. Becucci, A. Dolfi, M. R. Moncelli, F. T. Buoninsegni, *J. Electroanal. Chem.* **2001**, *504*, 1; b) W. Knoll, C. W. Frank, C. Heibel, R. Naumann, A. Offenhäuser, J. Rühe, E. K. Schmidt, W. W. Shen, A. Sinner, *Rev. Mol. Biotechnol.* **2000**, *74*, 137; c) E. K. Sinner, W. Knoll, *Curr. Opin. Chem. Biol.* **2001**, *5*, 705.
[13] B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, R. J. Pace, *Nature* **1997**, *387*, 580.
[14] V. L B. Braach-Maksvytis, B. Raguse, *J. Am. Chem. Soc.* **2000**, *122*, 9544.
[15] R. M. Epand, *Biopolymers* **1997**, *43*, 15.
[16] C. R. Woese, G. E. Fox, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5088.
[17] J. C. Mathai, G. D. Sprott, M. L. Zeidel, *J. Biol. Chem.* **2001**, *276*, 27266.
[18] D. J. Vanderah, C. P. Pham, S. K. Springer, V. Silin, C. W. Meuse, *Langmuir* **2000**, *16*, 6527.
[19] W. J. Kerr, G. G. Kirk, D. Middlemiss, *J. Organomet. Chem.* **1996**, *519*, 93.
[20] a) M. Ishiwatari, K. Yamada, R. Ishiwatari, *Chem. Lettl* **2002**, *3*, 206; b) P. G. Schouten, J. F. Van der Pol, J. W. Zwikker, W. Drenth, S. J. Picken, *Mol. Cryst. Liq. Cryst.* **1991**, *195*, 291.
[21] P. Wagner, M. Hegner, H. J. Güntherodt, G. Semenza, *Langmuir* **1995**, *11*, 3867.
[22] C. Peggion, F. Formaggio, C. Toniolo, L. Becucci, M. R. Moncelli, R. Guidelli, *Langmuir* **2001**, *17*, 6585.
[23] N. Bunjes, E. K. Schmidt, A. Jonczyk, F. Rippmann, D. Beyer, H. Ringsdorf, P. Gräber, W. Knoll, R. Naumann, *Langmuir* **1997**, *13*, 6188.
[24] B. Raguse, V. L B. Braach-Maksvytis, B. A. Cornell, L. G. King, P. D. D. Osman, R. J. Pace, L. Wieczorek, *Langmuir* **1998**, *14*, 648.
[25] G. Krishna, J. Schulte, B. A. Cornell, R. Pace, L. Wieczorek, P. D. Osman, *Langmuir* **2001**, *17*, 4858.
[26] H. Neurath, R. L. Hill, *The Proteins, Vol. 5*, Academic Press, New York, **1982**.