A Parallel Library of all Seven $A_2 + B_2 + C_2$ T_h Regioisomeric Hexakisadducts of Fullerene C_{60}: Inspiration from Werner's Octahedral Stereoisomerism**

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Compact multivalent molecules can be viewed as ideal cores for the synthesis of libraries with very high diversity.[1–3] Cubane and trimesic acid-based scaffolds,[2] among others,[3] have been proposed for this purpose. Extending functional valency of these cores to octahedral or higher symmetry objects, while maintaining control of the spatial directivity of branches derived from their anchor points, would give access to "spherical" diversity suitable for probing cell surface receptors and signaling pathways. Based on these premises, octahedral stereochemistry,[4, 5] originally proposed by Werner to explain the configuration of transition metal complexes, inspired us to develop approaches to novel scaffolds with potential applications in combinatorial chemistry.

With its six coordination sites, the octahedron has a much more complex stereochemistry than its tetrahedron counterpart, the latter possessing only configurational isomerism.[6] In fact, if six *different* groups are placed at the O_h sites, up to fifteen diastereomers can be generated.[5b] In a simpler case, the spatial arrangement of three pairs ($A_2 + B_2 + C_2$) of monodentate ligands at the O_h sites in transition metal complexes leads to the five diastereomers **1a–e** (Figure 1). In practice, this analysis is complicated by the lability of the ligands, hence bridging bi- or multidentate coordinating groups are required to gain control over molecular configuration.[5, 7] This aspect restricts the number of isomers that can be prepared and does not lead to the easy elaboration of libraries with different groups at all six O_h positions.

The high symmetry of C_{60} provides an opportunity to expand the diversity of molecular libraries by using a relatively compact (10-Å van der Waals radius "nanobead") and easily functionalizable spherical framework of thirty C=C bonds (Figure 2).[8, 9] Although this question has been addressed in part,[8–12] regio- and stereocontrolled additions to fullerenes remain a real challenge, and the functionalization of any of their double bonds, at will, is unthinkable at the present time.[8a] To simplify the problem by keeping the local symmetry to a maximum, one can make a useful analogy between C_{60} and Werner's octahedral framework (Figure 2, left).[9, 11] There are six symmetrically disposed double bonds situated at the vertices of an octahedron, which if functionalized selectively, give access to a variety of well-defined

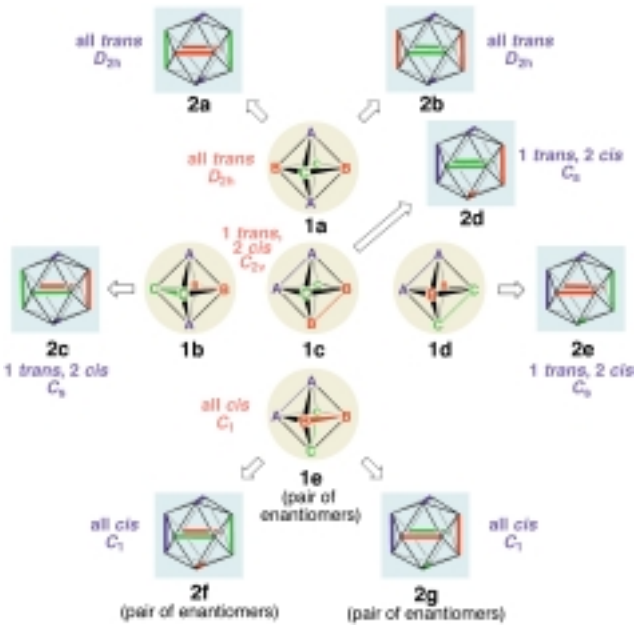

Figure 1. Stereoisomerism of $A_2 + B_2 + C_2$ addends within octahedral (**1a–e**) and pseudo-octahedral (T_h) topologies (**2a–g**).

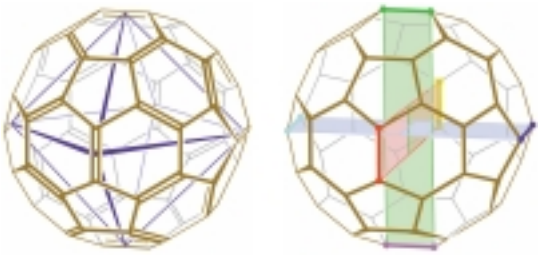

Figure 2. Octahedral and T_h topologies within C_{60}.

three-dimensional systems.[9, 13] Contrary to the freely rotating ligand-to-metal bonds in the octahedron, these bonds are fixed in space, being included in the three planes of symmetry that are implicitly part of the T_h point group (Figure 2, right).[6, 9a, 11] Consequently, the number of isomers that can arise from $A_2 + B_2 + C_2$ functionalization at these bonds increases to seven (Figure 1, **2a–g**), and goes up to thirty if all six adding groups are different (for symmetrical addends).

The methodology outlined here provides access to a small library of hexakisadducts (Figures 3 and 4) which have three pairs of adding groups ($A_2 + B_2 + C_2$ pattern) with topologies similar to those of the octahedral transition metal complexes, but with the added complexity of the T_h framework (Figure 1). In the octahedral model, there is only one possible diastereomer for each of the all-*trans* (**1a**) and all-*cis* (**1e**) config-

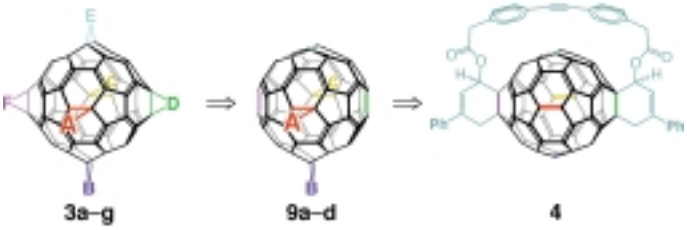

Figure 3. The "*mer*-3+3" retrosynthetic strategy to hexakisadducts **3a–g**.

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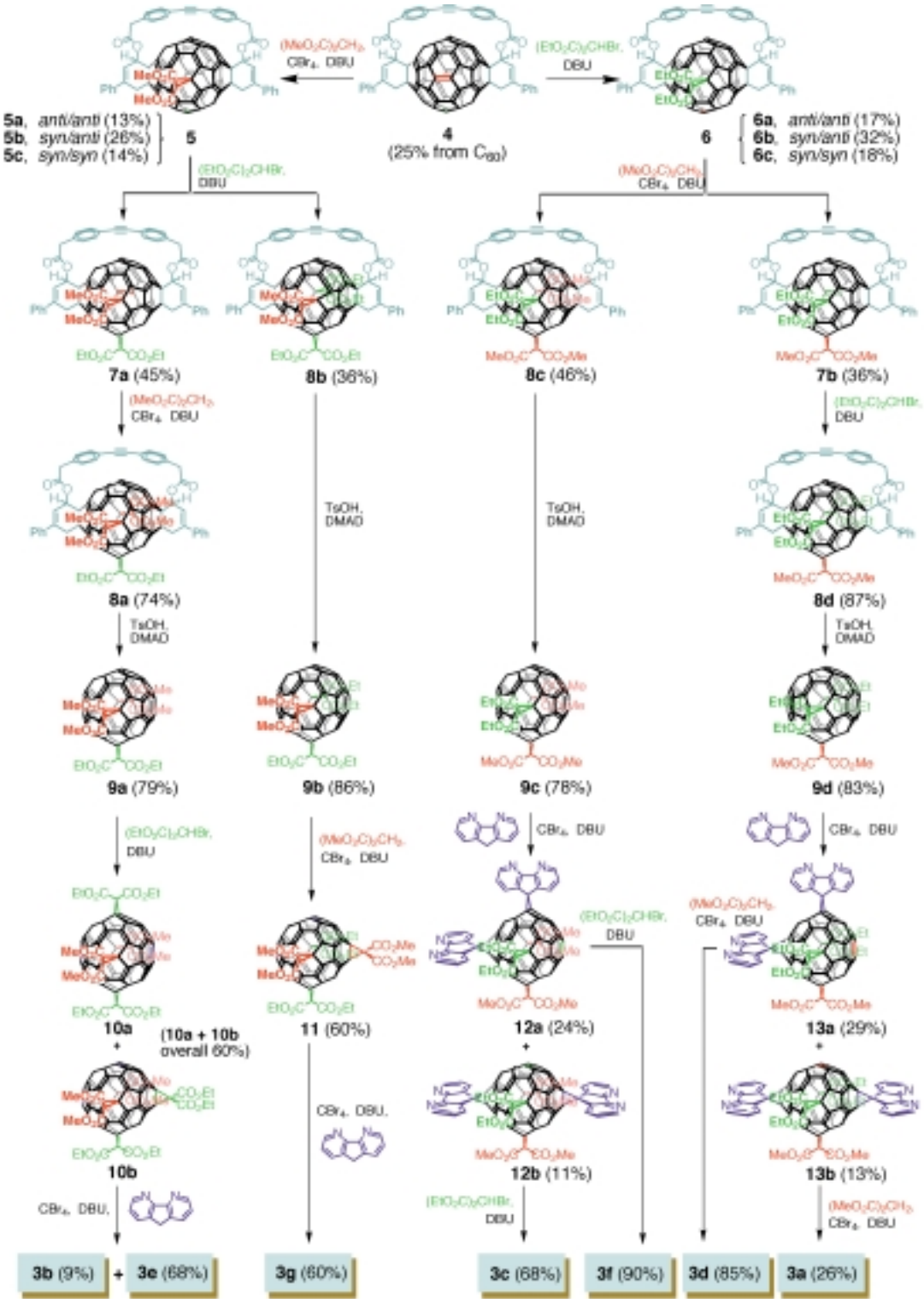
urations, while in the pseudo-octahedral (T_h) model, each of the all-*trans* and all-*cis* relationships give rise to a pair of compounds that are *regioisomers* or "permutational isomers"[14] (**1a** → **2a** and **2b**, **1e** → **2f** and **2g**).

The "*mer*-3+3" regiocontrol strategy[15] employed in the present synthesis is derived from the finding that the bridged *trans*-1 bisadduct **4** displays exquisite selectivity towards the sequential addition of three groups to its equatorial positions (Figure 3).[13] Within the scope of this work, four differentiated "*mer*"-trisadducts (**9a–d**) were needed as key intermediates (Scheme 1). It was also anticipated that the three *e*-face, *e*-edge, and *trans*-1 arranged addends of **9a–d** would direct further additions toward the remaining three *e*-positions.[13] However, the exact degree of regioselectivity achievable among the last three unoccupied C=C bonds at the pseudo-octahedral positions was not clear.



Scheme 1. Regioselective, parallel synthesis of the regioisomers **3a–g**.

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An economical execution of this strategy was needed to give rapid access to all seven diastereomers **3a–g** (Scheme 1). Thus parallel synthesis, which has proven to be a very efficient method to obtain large numbers of structurally related compounds, was applied here.[16] The three pairs of labeling addends, dimethyl malonate, diethyl malonate and 4,5-diazafluorene, were chosen for several reasons: they can all add to C_{60} following the Bingel–Hirsch regioselectivity, which allows them to be addressed especially well to equatorial positions.[11] In the ^{1}H NMR spectra, each of the three addends appears in a characteristic region, well separated from the signals of other groups. The large magnetic anisotropy from the 4,5-diazafluorenyl moiety has a significant influence on the chemical shifts of neighboring addends, and this property plays a crucial role in the confirmation of the relative stereochemistry in each isomer. In addition, the introduction of the very polar 4,5-diazafluorenyl groups helps overcome the separation problems caused by potentially unsatisfactory regioselectivity[13] in the late stages of the overall synthesis.

Monoaddition of a diethyl bromomalonate unit to the *trans*-1 bis-Diels–Alder adduct **4** (*syn*/*anti* mixture) gave one regioisomer **6** (Scheme 1).[13, 17] The two stereocenters at the cyclohexenyl rings imply that **6** consists of three possible diastereomers **6a** (*anti*/*anti*), **6b** (*syn*/*anti*) and **6c** (*syn*/*syn*), which were easily separated by column chromatography (CH_2Cl_2, SiO_2). To aid unambiguous characterization of the products in the next additions, the C_s-symmetrical compound **6a** was used in the following steps (**6b** and **6c** gave the same trimers **9c** and **9d** after the same addition and deprotection sequence). In earlier work,[13] we found that the second Bingel addition on **6** was also highly regioselective and gave the addition pattern that corresponds to **7b**. In the present case, we used 1.5 equivalents of dimethyl malonate to afford nearly equal amounts of the easily separable tetrakisadduct **7b** and pentakisadduct **8c** in high yield (82%). Only a trace ($\leq$5%) of an uncharacterizable brown fraction was obtained, again confirming the high selectivity of this reaction towards the "*mer*" triple-*e* position. The tetrakisadduct **7b** was further derivatized with diethyl bromomalonate to give exclusively the pentakisadduct **8d**. One-pot elimination/Diels–Alder/retro-Diels–Alder reaction[13, 18] removed the tethers on **8c** and **8d** to give the two trisadducts **9c** and **9d** in high deprotection yields (78% for **9c** and 83% for **9d**). Similarly, the other two heterotrisadducts **9a** and **9b** were obtained by simply switching the addition sequence on the *trans*-1 bisadduct **4**. It should be noted that these four trisadducts are only subtly different from each other, and that their rapid preparation is not possible by any other regiocontrol methodology.[9–12]

The four trisadducts **9a–d** served as the indispensable intermediates in the overall synthesis of all seven pseudo-octahedral diastereomers **3a–g**. The relative regioselectivity among the three remaining equatorial double bonds was studied in the final synthetic steps: earlier results[13] indicated that the fourth addition on these "*mer*"-trisadducts should greatly favor the triple-*e* position(s) (double *e*-edge, plus single *e*-face), which correspond to the "poles" of the sphere with respect to the original bis-Diels–Alder addition, rather than the double *e*-face site on the equator. Indeed, treatment of **9b** with one equivalent of dimethyl malonate under the Hirsch conditions[19] gave exclusively the expected triple-*e* adduct **11**. The last two *e*-double bonds were then "masked" by 4,5-diazafluorenyl groups to give the first isomer **3g** in this $A_2 + B_2 + C_2$ hexakisadduct series. Interestingly, when the C_{2v}-trisadduct **9a** was treated with one equivalent of diethyl bromomalonate under similar conditions, two tetrakisadducts **10a** and **10b** were obtained, which result from additions at both the double-*e*-face position and the triple-*e* positions, respectively. The ratio of these two inseparable isomers was 8:1 as determined by ^{1}H NMR spectroscopy. The reason for the reduced selectivity in this case is not clear, but one possible cause is the larger steric bulk of the diethyl bromomalonate anion compared to that of the dimethyl analogue. In any case, both isomers could be incorporated in the overall synthesis. Two 4,5-diazafluorenyl moieties were introduced in the final step to afford the desired products **3b** and **3e**. Although the tetrakisadduct isomers **10a** and **10b** were not separable, their resulting hexakisadducts **3b** and **3e** were easily separated by preparative TLC (SiO_2, CH_2Cl_2/MeOH 100/3.5) as a consequence of the different *cis* and *trans*-display of the two R_f-determining 4,5-diazafluorenyl groups.

In the final synthesis of the other four hexakisadducts **3a**, **3c**, **3d**, and **3f**, both *cis* and *trans* relationships of the two 4,5-diazafluorenyl groups on the pseudo-octahedral scaffold were required. To average out the relative amounts of the *trans* (**3c**) and *cis* (**3f**) isomers, we first treated the trisadduct **9c** with 1.4 equivalents of 4,5-diazafluorene under the Hirsch conditions. This approach afforded only the two possible pentaadducts **12a** (*cis*, 24%) and **12b** (*trans*, 11%), together with some tetrakisadduct (30%), recovered starting material **9c** (30%), and a trace of a higher adduct. These products were easily separated by column chromatography; the tetrakisadduct and starting material were recovered to prepare the pentakisadducts **12a**, **b** again. The last unoccupied octahedral position in **12a** (*cis*) was easily blocked with an excess of diethyl bromomalonate to give the hexakisadduct **3f**. The fraction of the "*trans*"-adduct **12b** was contaminated with about 10–20% of a compound that could not be characterized or removed by column chromatography. The "abnormal" addition pattern of this impurity is probably due to the much more reactive nature of the 4,5-diazafluorenyl anion compared to that of the malonate anions. After treatment of this mixed fraction with excess diethyl bromomalonate under standard conditions, the resulting bright yellow all-*e* hexakisadduct **3c** was easily isolated from the orange non-octahedral byproduct by preparative TLC (SiO_2, CH_2Cl_2/MeOH 100/4). Compounds **3a** and **3d** were prepared from the trisadduct **9d** using a similar reaction sequence. Starting from C_{60}, it took only 21 steps overall (on average three reactions per isomer) to obtain all seven diastereomers. No HPLC separation was necessary in this highly divergent synthesis.

The stereochemistry of all seven isomers can be unequivocally confirmed from their NMR spectra (Figure 4), and also from their synthetic history. In accord with the initial molecular design, the diamagnetic ring current from the large π surface of the 4,5-diazafluorenyl addend has a substantial

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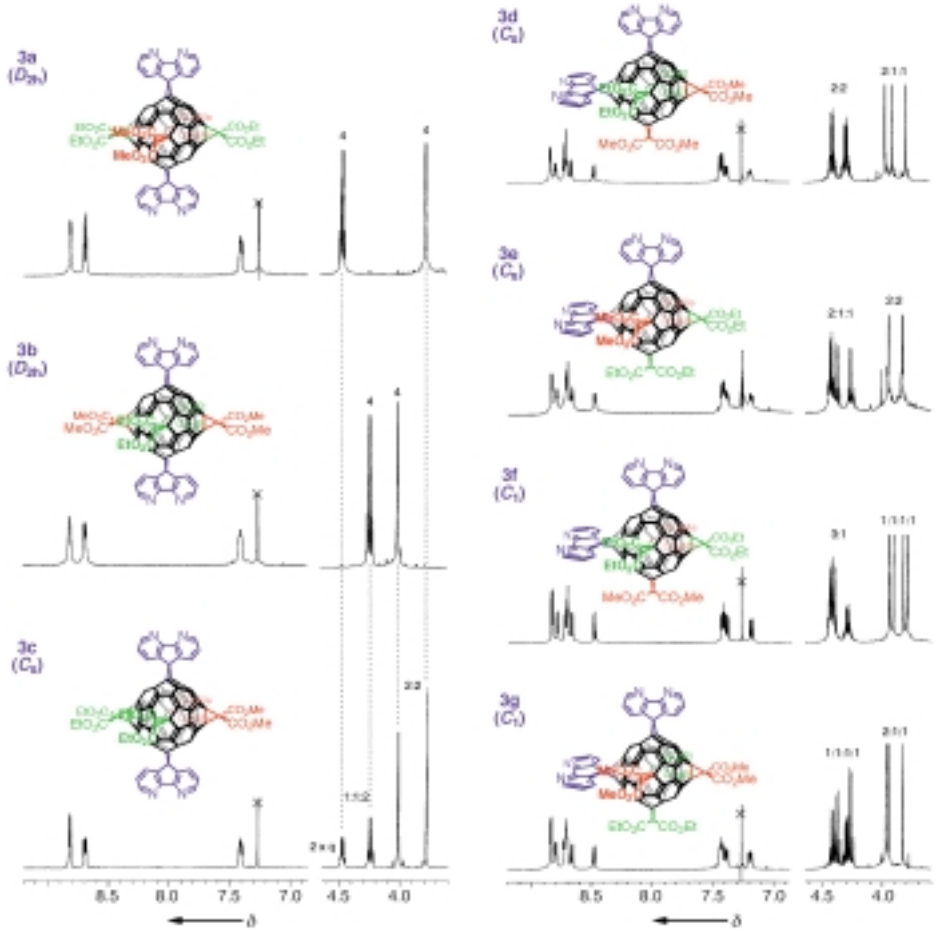

Figure 4. ^{1}H NMR spectra of the seven stereoisomers **3a**–**g** and their respective symmetry.

shielding effect on the chemical shifts of the protons of groups facing it. On the other hand, the ethyl and methyl ester groups both have a similar influence on their neighboring addends. Hence, the chemical shifts of the methylene protons (COOEt groups) or the methyl protons (COOMe groups) are particularly diagnostic in the determination of the relative position of each addend. This is especially important in distinguishing the two very similar C_1-symmetric all-*cis* compounds **3f** and **3g**. In **3f**, there are two methoxy groups facing the π surfaces of the 4,5-diazafluorenyl moieties, thus their proton signals are shifted to higher field relative to those of the other two methoxy groups in this molecule. On the other hand, there is only one ethoxy group facing the π surface of the 4,5-diazafluorenyl addend, its protons are shielded and thus appear at higher field than those of the other three ethoxy groups in **3f**. An exactly opposite effect is observed for the isomer **3g**.

Similarly, the all-*trans* geometric pair of compounds **3a** and **3b** cannot be distinguished by the number of signals in their relatively simple ^{1}H or ^{13}C NMR spectra because of their common D_{2h} symmetry. However, there is a dramatic difference in the 3.6–4.6 ppm region of their ^{1}H NMR spectra, which depends on whether or not the methoxy or ethoxy groups are facing the π surfaces of the 4,5-diazafluorenyl addends in these two isomers. Interestingly, compared with **3a**, the upfield shift ($\Delta\delta = 0.23$ ppm) of the ethoxy methylene protons in **3b** has exactly the same magnitude as the downfield shift of the methoxy protons. For the same reasons, the ^{1}H NMR spectrum of **3c** is almost the exact superposition of those of **3a** and **3b**.

The efficient synthesis of hexakisadducts **3a**–**g** demonstrates the utility of the stepwise "*mer*-3+3" regiocontrol strategy on C_{60}. In a broader sense, this work also provides an entry into another dimension of organic stereochemistry.[6, 20] Furthermore, access to a diverse and relatively complex display of functional groups on the spherical framework of C_{60} can be expected to afford new classes of compounds with biological properties.[8, 21]

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A Chip-Based Biosensor for the Functional Analysis of Single Ion Channels**

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The functional analysis of single ion channel proteins presents a serious bottleneck in the process of finding new pharmacologically active compounds. Single channel recording methods currently available (patch clamp,[1] black lipid membrane (BLM)[2]) are not suited for automation and miniaturization. However, new techniques such as combinatorial chemistry[3] and combinatorial genetics,[4] which produce large numbers of potential drugs and mutant proteins, respectively, demand efficient and reliable high-throughput screening (HTS) as well as low sample consumption.[5] In this context, monitoring the current of single ionotropic receptors with automated biosensor devices may result in new classes of highly sensitive screening tools.

Functional studies of single ion channel proteins require the reduction of electrical background noise to 10^{-13} – 10^{-12} A and consequently a high electrical insulation of the surrounding membrane patch ($R > 10^9\ \Omega$, "giga-seal") under conditions of low dielectric loss and low electrical capacitance.[1] Classically, tight seals are obtained manually by suction of small membrane patches (from cells or proteoliposomes) into glass micropipettes[6] or by "painting" of lipid bilayers across holes in teflon septa.[2, 7]

Single-channel recording systems suited for biosensor and HTS applications require a new approach. The self-positioning of unilamellar lipid vesicles on planar insulating diaphragms presents one solution. Since the surface of most native membrane vesicles and cells bears electrical charges,[8] properly directed electrical fields can provide precise electrophoretic positioning. However, efficient electrophoretic attraction of vesicles can only be realized for electrokinetic velocities v_{ek} (vesicle) $\gg 1\ \mu m\,s^{-1}$, which requires electric fields of several hundred volts per meter.[9] In a homogeneous field, such field strengths are only obtained under unfavorable conditions such as high voltages or very short electrode distances. Consequently, strongly inhomogeneous fields were used for positioning, which resulted in a focused movement of vesicles towards the point with the highest voltage gradient.

An inhomogeneous electrical field (Figure 1a) was created around a small aperture located in a thin insulating diaphragm (Figure 1b) separating two fluid compartments. By accessing both compartments with low impedance redox electrodes (Figure 1c) the main voltage drop after application of a potential V_C occurred within and near the aperture (Figure 1a). The resulting radially symmetrical field E directed the electrophoretic movement of charged objects to the spatially fixed aperture. The geometry of the aperture and the limitation of the applied voltage V_C to physiological potentials (< 200 mV, to avoid artifacts such as electroporation after seal formation) define the strength of the electric field. Optimal conditions for positioning were obtained by reducing the diaphragm thickness h to the minimum size that would provide sufficient stability and insulation ($h \approx 100$ nm) and by adaptation of the aperture diameter to the vesicle size ($d_A = 0.6 – 7\ \mu m$). Figure 1a illustrates that under optimal conditions the "electrophoretic trap" extends less than 20 μm into the buffer volume. Despite this short attractive range vesicles frequently enter the electrophoretic trap by convection during vesicle addition or by sedimentation within the small volume of the compartment (1 – 10 μL, Figure 2a).

The feasibility of electrophoretic focusing was demonstrated using negatively charged giant unilamellar vesicles (Figure 2b) and bare silicon nitride diaphragms. Within 1 – 10 s after the addition to the *cis* compartment (Figure 1c, upper side), vesicles were trapped and moved through the aperture. The resulting current modulations (Figure 2a) resembled Coulter-counter events ($V_c = 80$ mV).[10] Even vesicles larger

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