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FUNCTIONALITY OF POLYPEPTIDE BY INDUCTION OF SPECIFIC TERTIARY STRUCTURE

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ABSTRACT

Regular assemblies of peptide molecules to exhibit novel functionalities have been prepared in two different ways. One of them is a helix-bundle structure in which two or three hydrophobic α-helical peptides are connected together by a tie molecule. The helix-bundle structure formed an ion channel across lipid bilayer membrane at lower concentrations than a single-chain pepide.The other example is a two-dimensional assembly of amphiphilic peptide molecules carrying a chromophoric group. The assembly was an efficient photo-energy collector to generate photo-electron and to reduce cytochrome c.

INTRODUCTION

Proteins and polypeptides exhibit their specific functionalities on the basis of the specific tertiary structure. In order to develop peptidic materials having novel

functionalities, construction of a well-defined structure should be important. In the specific tertiary structure, functional groups are regularly arranged in space for cooperation to exhibit functionalities. In the present investigation, two different approaches were made to construct regular assembly of peptide molecules.

One of them is a helix-bundle structure in which two or three hydrophobic α-helical peptide molecules are connected together by a template or tie molecule, so-called template-assisted synthetic peptide (TASP) [1]. In the TASP, the assembled structure of helical chains is favored in conformational entropy and energy by connection to the template (tie) molecule. Here, the promotion of ion-channel formation by taking a bundle structure of α-helical peptides was investigated in lipid bilayer membrane.

The other is a two-dimensional assembly of amphiphilic peptide molecules carrying a chromophoric group. It has recently been reported that amphiphilic molecules construct vesicular assembly having a bilayer membrane structure in water [2]. Photo-physical and photo-chemical functions of the chromophoric regular assembly are worth investigation for creation of artificial photo-energy conversion system.

EXPERIMENTAL

Materials

Peptide molecules were synthesized by the conventional liquid-phase method. The synthesis of an amphiphilic compound (5cz18) has been reported previously [3]. The other amphiphilic compound (5cz18z) was prepared in a similar way.

Methods

CD fluorescence, and transient absorption spectroscopy were carried out on a JASCO J600 spectropolarimeter, a Hitachi MPF-4 fluorophotometer, and an Otsuka Electronics IMUC-7000 using a Xef excimer laser (351 nm, 54 mj), respectively.

Ion-channel formation by the peptide molecules was examined in BLM. A thin Teflon film (0.25-mm thickness) with an aperture of 0.2–0.3-mm diameter was clamped between two compartments in a Teflon trough. The aperture was precoated with a hexadecane/hexane (6/4 v/v) mixture. A soybean-lecithin membrane was formed according to the method reported by Montal and Muller [4]. The synthetic peptide was added to one of the two compartments which contain aqueous KCL solution (1M).

DMPC liposome was prepared by sonication of a lipid dispersion in a Tris-buffer solution. Liposomes of the amphiphilic compounds were prepared by an extruder method [5] or a sonication method.

RESULTS AND DISCUSSION

Template-Assisted Synthetic Peptide

Molecular Design for the Ion-Channel-Forming Peptides

Cell cytoplasm is partitioned from the outside by lipid membrane, which is essentially impermeable for ions and hydrophilic compounds. Ion-channel proteins and some antibiotics have been found to transport ions across the lipid membrane. The molecular structure of the ion-channel-forming compounds has been elucidated and an α–helix-bundle structure has been advocated to be essential [6]. For example, hydrophobic α-helical peptides have been shown to act as a voltage-dependent ion channel, which span across the lipid membrane and associate together to form the a-helix-bundle structure [7, 8]. Parallel orientation of the α-helix peptides in which the macrodipole moments of the α-helix peptides are aligned in the same direction has been assumed to be an active form of the ion channel. In the present study, in order to help realize this state, two or three chains of hydrophobic α-helical peptides were combined to a tie molecule to yield a complex peptide molecule (Figure 1). These compounds should aggregate themselves to form the α-helix bundle having the parallel orientation easily, and act as ion channel across lipid bilayer membrane.

Conformation

For hydrophobic α-helical peptide, an alternating hexadecapeptide of alanine and 2-aminoiso-butyric acid (Aib) having both terminals blocked, Boc-(Ala-Aib)$_8$-OMe, was synthesized. In the solid state, x-ray analysis revealed that Boc-(Ala-Aib)$_8$-OMe takes α-helical structure [9]. Conformation of the peptides in solution was investigated by CD spectroscopy (Figure 2). All the peptides in methanol showed CD spectrum of double-minimum pattern, indicating the occurrence of α-helical structure. The poly(Sar) part of 2αS and 3αS does not influence the conformation of the peptides, because CD spectra of 2αS and 3αS are similar to those of 2αZ and 3αZ, respectively. On the other hand, the α-helix content increased with increasing number of hexadecapeptide connected to the tie molecule in the order of $1\alpha < 2\alpha Z \approx 2\alpha S < 3\alpha Z \approx 3\alpha S$. Since the association of hexa-

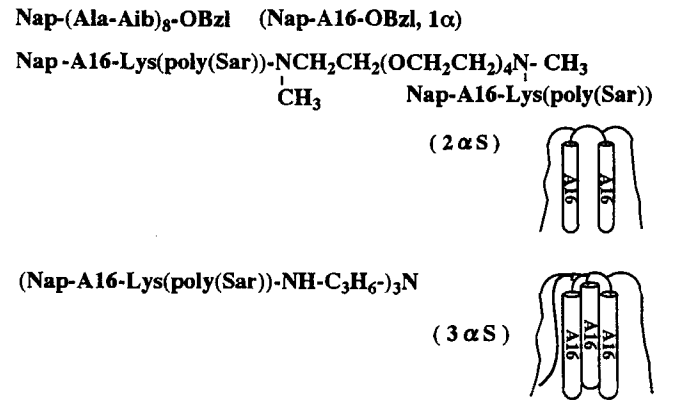

Figure 1. Molecular structure of the synthetic α–helical peptides.

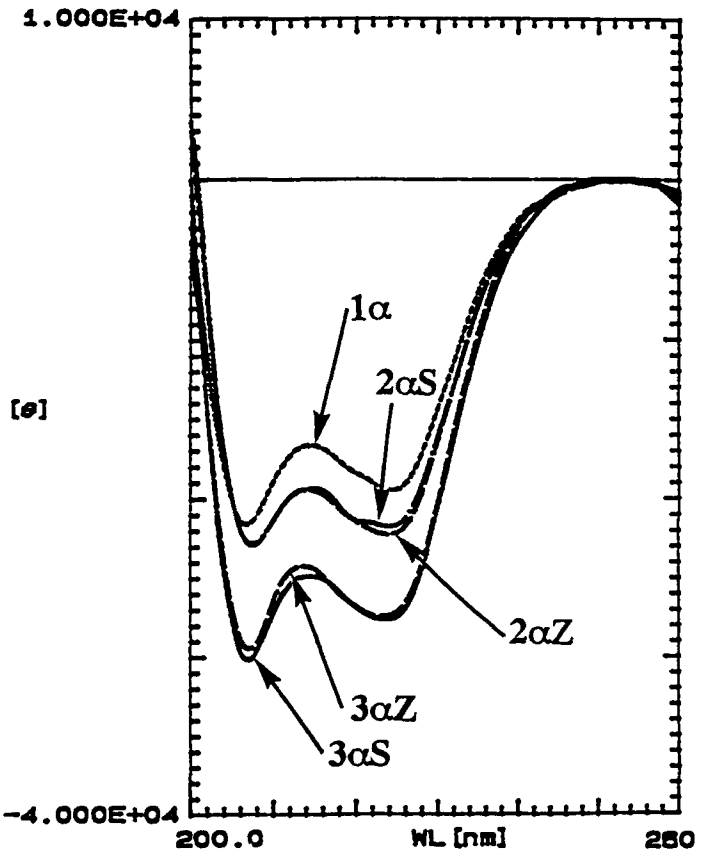

Figure 2. CD spectra of the synthetic complex peptides in methanol.

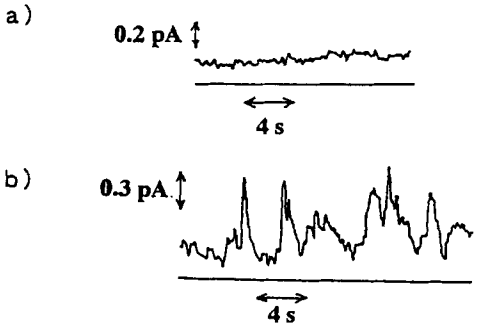

Figure 3. Current fluctuation at an applied voltage of 190 mv across the soybean-lecithin membrane in the presence of a) 1α (0.029 μm) and b) 3αs (0.026 μm).

decapeptide will become easier in this order, the α-helical conformation should be stabilized by the peptide association with parallel orientation.

The conformation of the peptides in dimyristoylphosphatidylcholine (DMPC) liposome was investigated by CD spectroscopy. The double-minimum pattern of CD spectra were observed with 2αS and 3αS with the molar elipticity at 235 nm stronger than that at 208 nm, suggesting an association of α-helical peptides in lipid membrane [10]. A helix-bundle structure of these peptides should be formed in the lipid membrane. On the other hand, the addition of 2αZ and 3αZ induced aggregation of liposome. The partition of the hydrophobic hexadecapeptides to the lipid membrane makes the membrane surface hydrophobic and disturbs the membrane structure, leading to aggregation of liposomes. The poly(Sar) chains of 2αS and 3αS are very effective to give the amphiphilicity to the complex peptides, so that these peptides are taken into lipid membrane without inducing aggregation of liposomes. These characteristics of 2αS and 3αS are promising for ion-channel formation.

Ion-Channel Formation

Ion-channel formation in lipid membrane can be detected by current fluctuation upon application of electric voltage across the lipid membrane. Current across BLM was measured with changing applied voltage and concentration of the peptides added. The addition of 3αS induced current fluctuation at lower concentration than 1a (Figure 3) or 2αS, suggesting an easy formation of ion channel by 3αS.

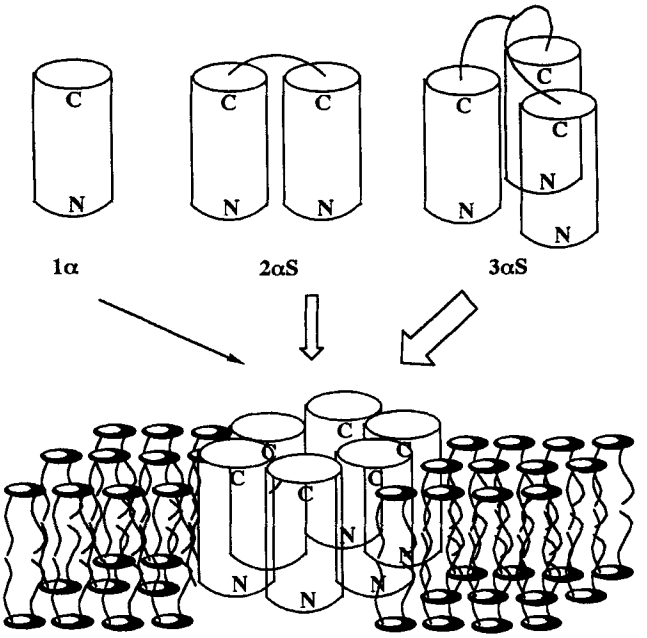

Figure 4. Schematic presentation of the complex peptides partitioned to phospholipid bilayer membrane. The thicker arrow mark represents the easier formation of helix-bundle structure in lipid membrane.

Interestingly, the electric current induced by 3αS was not clearly dependent on the applied voltage across the lipid membrane. These findings support the view that a bundle structure of α-helical peptide rods in parallel orientation leads to ion-channel formation , and that the TASP 3as is favorable for association to form ion channel at low applied voltage as illustrated in Figure 4.

Two-Dimensional Assembly of Amphiphilic Peptide Molecules

Molecular Design for Photo-Energy-Harvesting System

Dialkylammonium-type amphiphiles have been reported to form vesicular assembly in water [2]. When a chromophoric group is connected to each amphiphile, two-dimensional assembly of the chromophoric amphiphiles will be an excellent model for photo-energy-harvesting system because of high density of chromophoric groups regularly arranged in the assembly. In the chromophoric assembly, photo-energy will be migrated effectively among the chromophoric groups to an accepter molecule. For photo-energy-harvesting system, the following conditions

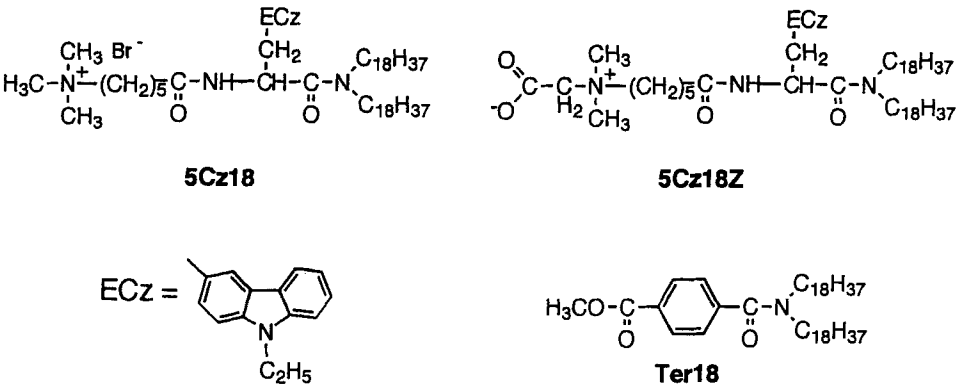

Figure 5. Molecular structure of the amphiphiles carrying the ECz group (5Cz18 and 5Cz18Z) and the quencher.

should be fulfilled: i) efficient photo-energy migration among chromophores, ii) high yield of electron transfer, and iii) stabilization of the charge-separated state.

Then, dialkylammonium-type amphiphiles (5Cz18 and 5Cz18Z) carrying l-3-(3-*N*-ethylcarbaz-olyl)alanine (Figure 5) were considered. It has been reported that neither ground-state interaction nor excimer formation occurs in the bilayer membrane of dialkylammonium-type amphiphiles carrying *N*-ethylcarbazolyl (ECz) group [3]. The absence of excimer formation guarantees the absence of photo-energy-trapping site [11]. In the present investigation, photo-energy migration was estimated by quenching experiment by using methyl terephthaloyldialkylamide (Ter 18, Figure 5) as an energy acceptor. The stability of the radical cation produced by laser-light irradiation was estimated by transient absorption spectroscopy. In addition, the photo-reduction of cytochrome c by the chromophoric assembly was investigated.

Characterization of the Molecular Assembly Of 5Cz18

It has been reported on the basis of TEM observation that an aqueous dispersion of 5Cz18 turned to an aqueous dispersion of small vesicles of diameters between 200 and 800 Å after a brief sonication [3]. DSC analysis of the vesicles showed an endothermic peak at 25.4°C, which corresponds to a gel–liquid-crystal phase transition of amphiphile bilayer membrane. 5Cz18Z, which is a Zwitter-ion-type amphiphile, similarly formed vesicles in water, and the phase-transition temperature was observed at 26.7°C.

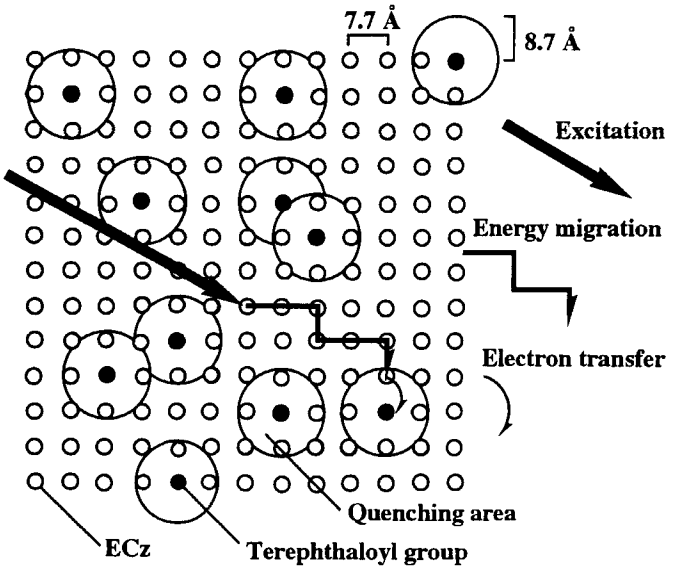

Figure 6. Simulation of the fluorescence quenching process in the mixed membrane of 5Cz18 and Ter18. ECz groups and terephthaloyl groups are placed on a square lattice with aspacing of 7.7 Å. The interaction distance between an excited ECz group and a terephthaloyl group is 8.7 Å.

Photo-Energy Migration in the Amphiphile Membrane

Fluorescence spectra of 5Cz18 assembly in water were measured with varying concentrations of Ter18 and at two different temperatures. The fluorescence quenching rate at 18°C, which is below the phase-transition temperature of 25.4°C, is larger than that at 40°C. The bilayer membrane structure should facililate energy migration to enhance the quenching rate. Therefore, the high quenching rate in a gel-state membrane may be due to efficient migration of excitation energy among chromophores in the membrane.

Fluorescence quenching in the bilayer membrane composed of 5Cz18 and Ter18 was simulated under an assumption that ECz groups and terephthaloyl groups reside on square lattice points with a spacing of 7.7 Å (Figure 6), which is taken from the x-ray data of the crystalline structure of dioctadecyldimethylammonium bromide bilayer membrane [12]. Since the interaction distance between the excited ECz group and the terephthaloyl group has been reported to be 8.7 Å [13], the excited ECz group is quenched when one of the four nearest sites from the ECz group is occupied by a terephthaloyl group. The results of simulation are shown in

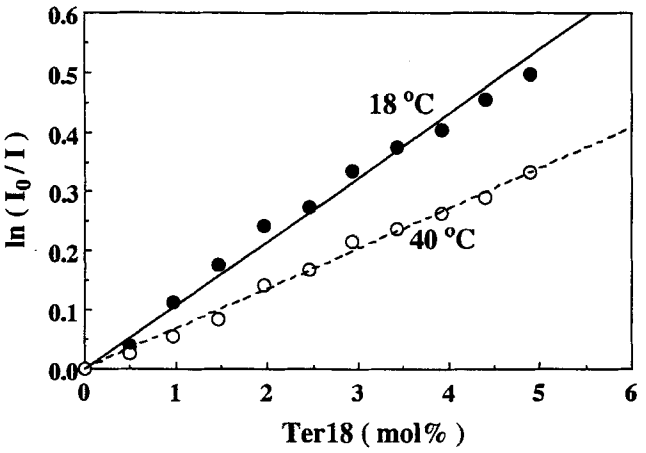

Figure 7. Perrin plot of 5Cz18 fluorescence quenching with Ter18 in the 5Cz18 /Ter18 mixed bilayer membrane. The experimental data at 18°C (●) and 40°C (○) are shown together with the results of computer simulation taking w/k_f to be 23 (—) and 9 (····).

Figure 7, in which the values of W/k_f (23 at 18°C and 9 at 40°C; W and k_f represent the rate of energy migration and emission, respectively) and the quantum yield of 5Cz18 in bilayer membrane (5.0 at 18°C and 1.9 at 40°C) were appropriately taken. The experimental results are in excellent agreement with the simulation curves. Consequently, the frequency of energy migration during the life time of excited ECz group were determined to be 5.0 times at 18°C and 1.9 times at 40°C.

Hole Transfer in Amphiphile Membrane

When the bilayer membrane containing 5Cz18 is irradiated by an excimer-laser beam, a biphotonic process may occur to yield radical cation ($ECz^{+\cdot}$) by ejecting an electron. The produced radical cation (hole) hops among the ecz groups under coulombic attraction from a radical anion which is produced by trapping the ejected electron. It has been reported that the ejected electron is trapped to a site about 20 Å apart from the parent radical cation [14].

Figure 8 shows transient absorption spectra of 5Cz18 bilayer membrane at 18°C and the change of absorbance at 780 nm with time. The time course was fitted by a single exponential curve with the life time of $ECz^{+\cdot}$ being 130 μs. This is relatively a long life time compared with a few μs of the ECz group in micelle reported by Nakamura *et al.* [15]. A possible explanation may be the existence of

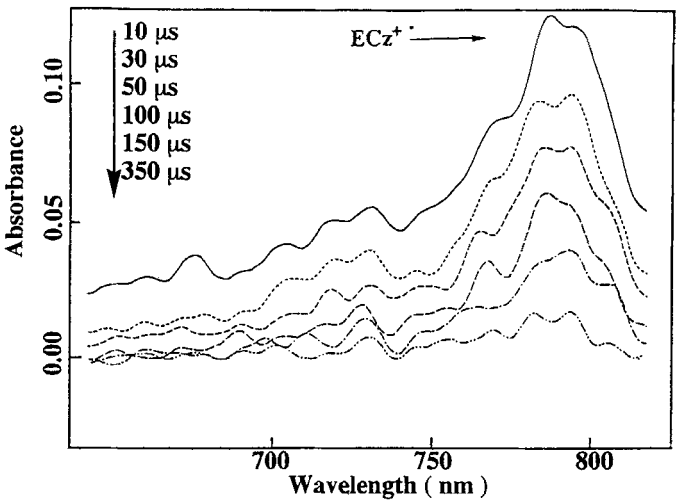

Figure 8. Transient absorption spectra of 5Cz18 bilayer membrane at 18°C at different time lapses after laser irradiation.

carbonyl groups in the present assembly. The electron ejected from the ECz group may be solvated initially, which will then be stabilized by trapping by the carbonyl group.

On the other hand, the life time of $ECz^{+\cdot}$ was 40 μs at 40°C. The short life time above T_c may be due to the lateral diffusion of the amphiphiles in the bilayer membrane, facilitating encounter of $ECz^{+\cdot}$ with a radical anion. Therefore, the rate of hole transfer, if it occurs below T_c, should be comparable to the rate of lateral diffusion of the amphiphiles (10^{-8} cm^2/s). The rate of hole transfer in the poly-(vinylcarbazole) film has been reported to be 10^{-5} cm^2/s [16]. Thus, the rate of hole transfer in the bilayer membrane is remarkably low, suggesting the stabilization of radical cation in the bilayer membrane.

Photo-Electron Transfer to Cytochrome C

Photo-reduction of cytochrome c by the ECz-containing bilayer membranes was investigated. It was found in the binding experiment that cytochrome c was partitioned to the 5Cz18Z bilayer membrane more easily than to the 5Cz18 bilayer membrane. Since cytochrome c bears positive charges at neutral pH region, it might have higher affinity toward the electrically neutral bilayer membrane (5cz18z) than the positively charged bilayer membrane (5cz18).

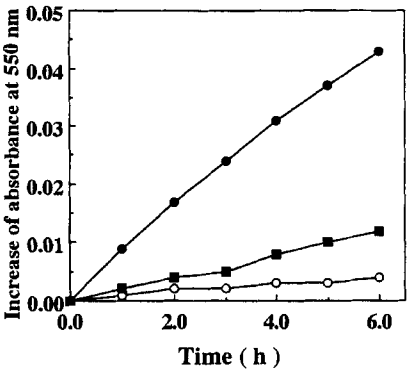

Figure 9. Reduction of cytochrome c monitored by the increasing absorbance at 550 nm. (●) 5Cz18Z/ cytochrome c/triethanolamine with irradiation, (○) 5Cz18Z/cytochrome c/triethanolamine without irradiation, (■) cytochrome c/ triethanolamine with irradiation.

Triethanolamine was added as an electron donor to the bilayer membrane system for cytochrome c reduction. Cytochrome c was scarcely reduced by 5Cz18Z membrane when the system was kept in dark. However, upon irradiation cytochrome c was effectively reduced (Figure 9). Two kinds of mechanisms are considered for the photo-reduction; i) photo-electron transfer from ECz group to cytochrome c and ii) electron transfer from triethanolamine to the heme group of cytochrome c which is activated by energy transfer from the excited ECz group. Further investigation is necessary for the mechanism to be firm.

CONCLUSION

In the present investigation, two or three hydrophobic α-helical peptides were connected together by a template (tie) molecule to construct a helix-bundle structure. The formation of helix-bundle structure of the complex peptide in phospholipid membrane was shown by CDspectroscopy, and the assembly acted effectively as ion channel. Therefore, the TASP method was found to be useful as a clue to solve the protein-folding mechanism.

Amphiphilic molecules carrying a chromophoric group were assembled to form bilayer membrane. The chromophoric groups were kept regularly in the

bilayer membrane to permit effective photo-energy migration and to stabilize radical cation produced. The 5Cz18Z bilayer membrane reduced cytochrome c effectively by photo-irradiation. The two-dimensional assembly was also found to be useful for establishment of regular arrangement of functional groups to elicit new functionalities

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