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Mechanosensitive Membrane Probes

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Abstract: *This article assembles pertinent insights behind the concept of planarizable push-pull probes. As a response to the planarization of their polarized ground state, a red shift of their excitation maximum is expected to report on either the disorder, the tension or the potential of biomembranes. The combination of chromophore planarization and polarization contributes to various, usually more complex processes in nature. Examples include the color change of crabs or lobsters during cooking or the chemistry of vision, particularly color vision. The summary of lessons from nature is followed by an overview of mechanosensitive organic materials. Although often twisted and sometimes also polarized, their change of color under pressure usually originates from changes in their crystal packing. Intriguing exceptions include the planarization*

of several elegantly twisted phenylethynyl oligomers and polymers. Also mechanosensitive probes in plastics usually respond to stretching by disassembly. True ground-state planarization in response to molecular recognition is best exemplified with the binding of thoughtfully twisted cationic polythiophenes to single- and double-stranded oligonucleotides. Molecular rotors, en vogue as viscosity sensors in cells, operate by deplanarization of the first excited state. Pertinent recent examples are described, focusing on λ-ratiometry and intracellular targeting. Complementary to planarization of the ground state with twisted push-pull probes, molecular rotors report on environmental changes with quenching or shifts in emission rather than absorption. The labeling of mechanosensitive channels is discussed as bioengineering approach to bypass the challenge to create molecular mechanosensitivity and use biological systems instead to sense membrane tension. With planarizable push-pull probes, this challenge is met not with twistome screening but with "fluorescent flippers," a new concept to insert large and bright monomers into oligomeric probes to really feel the environment and shine also when twisted out of conjugation.

Introduction

The dramatic change of color of lobsters, crabs or shrimps during cooking does not originate from molecular transformations but exclusively from supramolecular chemistry (Figure 1).[1] The chromophore, astaxanthin **1**, remains the same (Figure 2). What is observed is the thermal denaturation of β-crustacyanin, the lipocalin β-barrel that hosts astaxanthin. Without barrel, after denaturation, astaxanthin shows the characteristic color of a carotenoid. Within the confined

space of the barrel, the strongly twisted polyene is fully planarized. Moreover, a polarity gradient in the environment further polarizes the extended, planarized

Figure 1. Colorful impressions from the fishmarket.

polyene chromophore. Simply speaking, in living lobster, the astaxantin chromophore is planarized and polarized until it turns blue. In reality, origin of the lobster shift is necessarily more complex, including significant contributions from dimerization and oligomerization. Nevertheless, such contributions from coupled chromophore planarization and polarization to spectral tuning can be found quite often in nature. With fluorescent probes, the same coupled process is essentially unexplored. Very recently, we have proposed that the combination of planarization and polarization could provide a conceptually new approach to fluorescent membrane probes and beyond.[2,3] The mechanosensitivity of planarizable push-pull probes should be ideal to respond to changes in the disorder of lipid bilayer membranes,[3,4] including the so far poorly detectable tension.[5] Macrodipole-potential interactions[6] could possibly suffice to planarize twisted push-pull fluorophores and thus provide a conceptually new approach to visualize membrane potentials with the ultrafast response needed for neural imaging.[7] In the following, we aim to put the concept of planarizable push-pull probes into context and summarize briefly inspirational insights from biology to mechanosensitive organic materials.

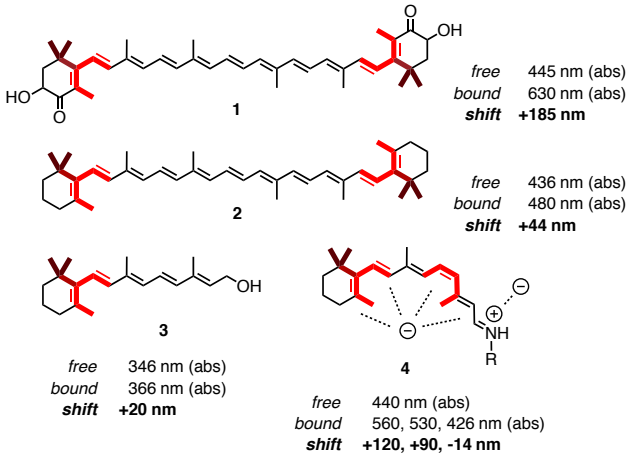

Figure 2. Examples from biology for the coupled planarization and polarization of chromophores include astaxantin **1** bound to β-crustacyanine and rhodopsin **4** with 11-*cis* retinal bound via a reversible protonated Schiff base to opsin. Red shifts upon planarization of terpenoids without complications from additional polarization have been confirmed for the encapsulation of β-carotene **2** within artificial β-barrels and the binding of retinol **3** to β-lactoglobulin.

Lessons from Nature

In solution, astaxanthin **1** exhibits an absorption maximum at λ_{abs} = 445 nm that accounts for the characteristic deep orange color of carotenoids with slightly extended conjugation compared to β-carotene (Figure 2).[1] In living lobster, crab or shrimp, the absorption of the same chromophore is at λ_{abs} = 630 nm. The crystal structure of astaxanthin **1** bound to β-crustacyanin demonstrated that full planarization of the twisted polyene contributes to the "Lobster shift" of at $\Delta\lambda_{abs}$ = +180 nm. In carotenoids, steric interference from the methyl substituents generally twists the β-ionone rings of the plane of the polyene chain. Ring-chain planarization then can occur in s-*cis* or s-*trans* conformation. Besides this major ring-chain twist, less impressive steric hindrance also gradually deplanarizes the polyene chain. Astaxanthin polarization by the environment within the β-barrel receptor further adds to the spectacular Lobster shift. Besides this combination of planarization

and polarization, significant higher-order contributions from dimerization and subsequent oligomerization should be ignored.

With synthetic systems, the planarization of carotenoids in monomeric form has been realized early on.[8] In solution, β-carotene **2** absorbs at λ_{abs} = 436 nm. Encapsulated within an artificial β-barrel with topologically matching hydrophobic interior and a hydrophilic surface, the same chromophore absorbed at λ_{abs} = 480 nm. This is one of the few examples in the literature where significant red shifts in synthetic systems can be attributed exclusively and with relatively high certainty to the planarization of a twisted chromophore in monomeric form. Intriguingly, this ground-state planarization is achieved with a chromophore that is composed of carbon and hydrogen atoms only, free of other functional groups. Encapsulated within an artificial β-barrel, chromophore polarization is unlikely to contribute to the observed bathochromic effect.

In biology, a similar red shifts $\Delta\lambda_{abs}$ = +20 nm to λ_{abs} = 366 nm with increase in vibrational finestructure have been reported for the planarization of monomeric vitamin A **3** within a biological β-barrel, named β-lactoglobulin.[9] Crystal structures of the complex confirmed full ring-chain planarization of the diterpene chromophore in s-*cis* conformation.

Chromophore planarization and polarization contributes significantly to the chemistry of vision.[10] The receptors accounting for the absorption of red, green and blue light contain structurally identical polyene chromophores **4**, i.e., the protonated Schiff bases of 11-*cis*-retinal. Their different absorptions originate from differences in planarization of the polyene chromophores and differences in polarization by charges placed along the strongly twisted scaffold. Model studies on the latter can be traced back to Nakanishi's pioneering external point charge model focusing on shifts caused by negative charges near the twisted π-system[11] Complementary examples for spectral tuning of planar, twist-free chromophores with nearby charges include green, red and cyan fluorescent proteins[12] or the recent introduction of ionpair-π interactions.[13] It is important to highlight that effects beyond planarization and polarization contribute to color vision, and that the origin of the opsin shift still remains under debate.[10]

For biological examples of the deplanarization of chromophores by chemical transformations rather than conformational changes, riboflavins, bent out of plane upon reduction, could be mentioned.[14] A great example for the same process comes from plant pigments (Figure 3).[15] With λ_{abs} < 340 nm, anthocyanines such as **5** are colorless in solution because hydration breaks the conjugation between the aromatic systems. In plant flowers, this hydration is prevented. Co-pigmentation in socially sorted π-stacks with electron-rich partners, mainly flavonoids, shifts the absorption of the planarized anthocyanine **6** by $\Delta\lambda_{abs}$ > +180 nm to λ_{abs} ~ 520 nm. Hydration of anthocyanine **6** in the π-stack is prevented because deplanarization would destroy the π-stack. Again it has to be cautioned that the molecular origins of plant co-pigmentation are much more complex and diverse, highly pH dependent and often including also coordination to metal cations.

Mechanosensitive Materials

One of the most popular system in mechanosensitive materials operates with a planarizing transformation similar to plant co-

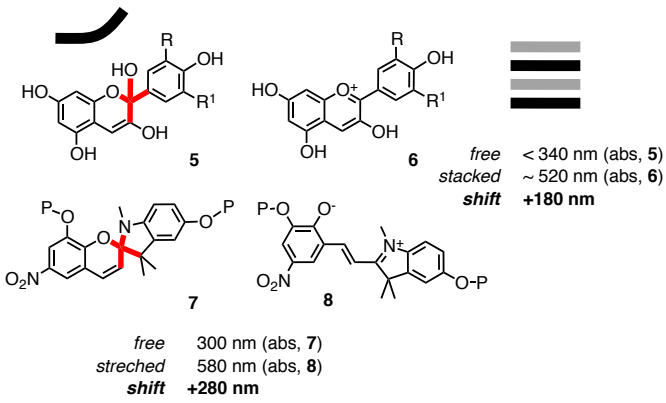

Figure 3. Examples for chromophore planarization by chemical transformations include dehydration of colorless hemiacetal **5** into the colored anthocyanine **6**, driven by donor-acceptor π-stacking, i.e. the co-pigmentation of plant flowers, and the opening of the colorless spiropyrane **7** into the colored merocyanine **8** in organic materials under stress.

pigmentation.[16] Like hydrated anthocyanine **5**, the colorless spiropyrane **7** with λ_{abs} = 300 nm contains a central tetrahedral carbon that disconnects and twists the aromatic systems out of plane (Figure 3). Transformation into the merocyanine **8** planarizes and polarizes the system into a fully conjugated push-pull fluorophore. The result is a spectacular $\Delta\lambda_{abs}$ = +280 nm to λ_{abs} = 580 nm. Polymers **7** with spiropyran mechanophores covalently engineered into their main chain report stress by stretching with the appearance of purple color. Several other fabulous examples for transformative mechanophores exist, the used reaction are often irreversible.[16]

Many mechanosensitive materials contain twisted aromatic systems, sometimes also polarized aromatics.[17,18] However, changes in absorption or emission in response to pressure, stretching, grinding and so on that clearly and exclusively originate from monomer planarization are difficult to identify (see below). The closest to clean spectral tuning in response to planarization under pressure comes an early landmark report from the Swager group (Figure 4).[19] Phenylethynyl oligomers and polymers such as **9** can equilibrate between conformers with and without conjugation. Co-planar orientation of the phenyls affords full conjugation, perpendicular orientation of the phenyls breaks conjugation at the triple bond. To break all conjugation, phenyl rings in **9** were equipped alternately with alkyl chains pointing to one side and triethyleneglycol chains pointing to opposite sides. Placed at the air-water interface of a Langmuir trough, the hydrophobic alkyl tails orient into the air and turn the phenyl rings into perpendicular orientation with respect to the interface. The hydrophilic trietyleneglycol chains enter into the water and orient the rings parallel to the interface. As a result, the phenylethynyl polymer **9** will be twisted out of conjugation. Application of lateral pressure will force the hydrophilic rings to rotate into perpendicular orientation and thus enforce conjugation. Consistent with increasing conjugation in the ground state, a red shift of $\Delta\lambda_{abs}$ = +37 nm to λ_{abs} = 458 nm was found in response to mechanical pressure applied in the Langmuir trough. A weaker red shift of $\Delta\lambda_{em}$ = +17 nm to λ_{em} = 467 nm was seen in the emission spectrum. A new band at λ_{em} = 535 nm, presumably originating from excimers, and a strong decrease in emission intensity illustrated that an

interpretation of the observed spectral response based on planarization only would oversimplify the situation.

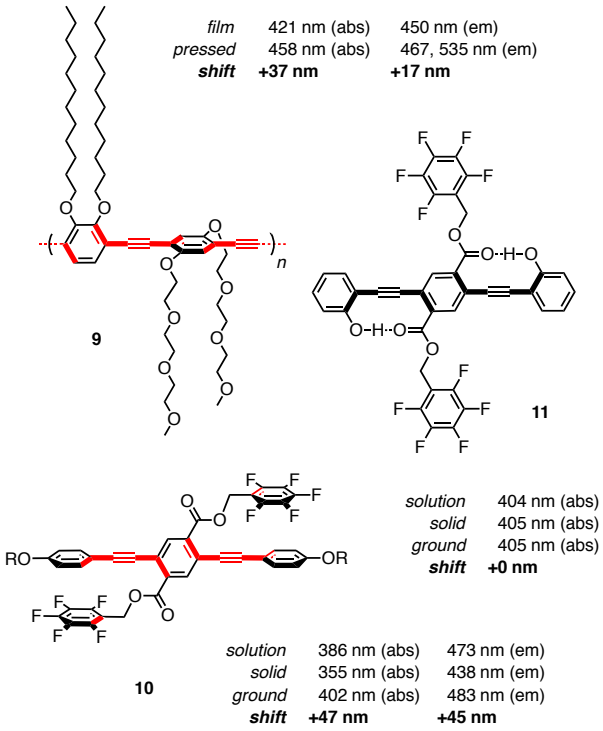

Figure 4. Red shifts obtained for the planarization of twisted polymer **9** by lateral pressure in the Langmuir trough and twisted oligomer **10** by grinding. No shifts were found for the planar control **11** upon grinding.

Very recently, these exciting results with mechanosensitive phenylethynyl polymers **9** at the air-water interface could be complemented with twisted phenylethynyl oligomers **10** in the solid.[20] The key challenge, that is how to twist phenylethynyls out of conjugation, was addressed with a very original approach. The central ring was equipped with π-acidic pentafluorophenyl rings. Face-to-face donor-acceptor stacking twists the peripheral π-basic rings of the oligophenylethynyl scaffold out of conjugation. In control molecule **11**, this deplanarizing face-to-face stacking is overcompensated by planarizing intermolecular hydrogen bonds. In solution, the planar conformer **11** absorbs at λ_{abs} = 404 nm, the twisted conformer **10** at λ_{abs} = 386 nm. In the crystal, the absorption of the twisted conformer **10** shifts with λ_{abs} = 355 nm further to the blue. This shift

suggested that the deplanarization of oligomer **10** increases in the solid state and is thus only partial in solution. Unchanged bathochromic absorption of control **11** in the crystal demonstrates full conjugation in solid and solution. Crystal structures of **10** and **11** fully confirm the expected twisted and planar conformations. Upon grinding, the absorption of the twisted oligomer **10** shifts by $\Delta\lambda_{abs}$ = +47 nm to the red. The resulting λ_{abs} = 402 nm would be consistent with full planarization in response to mechanical stimulation. This result is intriguing, implying the mechanical destruction of the intramolecular face-to-face stacks in **10**. However, results described in the following caution that interpretations based on planarization in the solid remain uncertain without crystal structures, changes in packing seem more frequent and more influential.

Mechanochromic materials have received much recent attention and have also been reviewed previously.[17,18] Deplanarized chromophores occur most often, push-pull chromophores as well in a few rare examples for the combination of planarization and polarization. However, deplanarization appears important mostly to assure loose packing in the solid, and changes in packing rather than planarization usually account for mechanosensitivity. Pyrene **12** is an early highlight in the field (Figure 5).[21] The approach builds elegantly on the mismatch of repeat distances in face-to-face π-stacks (~3.4 Å) and hydrogen-bonded chains (~5.0 Å, Figure 5A). The same mismatch strategy has been used in functional systems to build ligand-gated ion channels and photosystems in membranes and on solid surfaces.[22] In solution, the phenyl substituents of pyrene **12** are twisted out of plane because of steric interference of the hydrogens at the periphery of the aromatic planes. In the solid, the absorption shifts further to the blue, indicating full deplanarization accomplished by the nearby hydrogen-bonded chains. Mechanosensitivity was demonstrated by a red shift of $\Delta\lambda_{abs}$ = +50 nm to λ_{abs} = 400 nm under pressure. This shift was mainly attributed to changes in crystal packing rather than planarization.

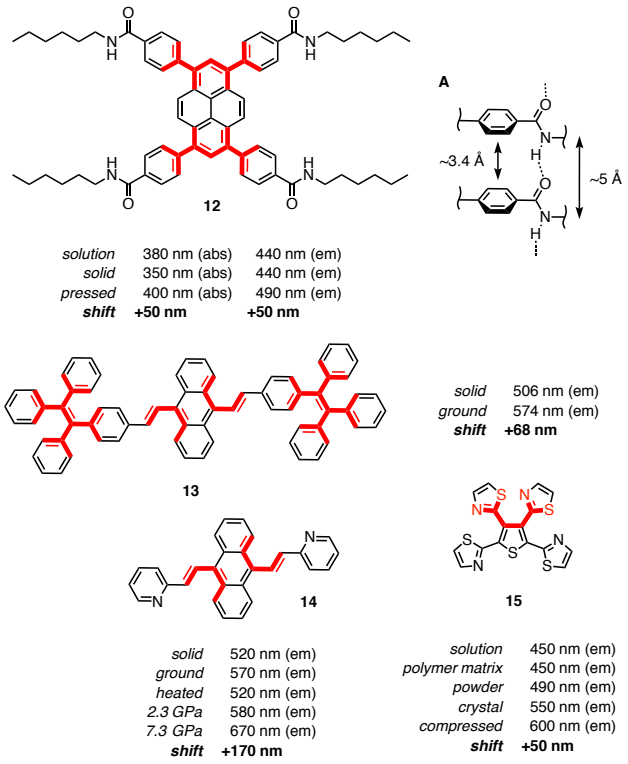

Figure 5. Mechanosensitive materials made from twisted monomers, with red shifts obtained in response to mechanical stress. A. Intrinsic topological mismatch of π-π interactions and hydrogen bonds.

The beautifully twisted mechanophore **13** is exceptional also because, like the planarizable β-carotene **2**, it is composed of carbons and hydrogens only (Figures 2 and 5).[17] The twisted double bonds in **13** originate from the diarylethene motif. Related to the chemistry of vision,[10] the sensitivity of stilbenes and their derivatives to the environment has been explored for decades.[23] They have been deplanarized by intramolecular steric constraints,[23] in carefully crafted molecular capsules,[24] metal-organic frameworks,[25] and so on. Attempts to reverse mechanical deplanarization have focused on carbon dioxide to compete for the same confined space in metal-organic frameworks.[25] Strongly twisted stilbene derivative have attracted much attention for photoswitching, photoinduced rotations[26] and photochromic cyclizations.[27] Deplanarization of the double bonds in mechanophore **13** has been used to reduce conjugation and to assure loose crystal packing. The large shift $\Delta\lambda_{em}$ = +68 nm to λ_{em} = 574 nm obtained under pressure originates presumably from changes in packing with little contribution from

planarization. Much larger versions of similar motifs have been reported to shift mechanosensitvity more to the red.[17]

Anthracene **14** represents a smaller version of the same motif.[28] This mechanophore is important because of the large $\Delta\lambda_{em}$ = +170 nm obtained in emission as well as the availability of crystal structures at different pressure. The results confirm that the significant mechanochromism originates from changes in crystal packing. With increasing pressure, the anthracenes planes, originally far apart, are coming closer and closer towards perfect face-to-face π-stacks in the crystal. The pyridine side chains are originally twisted out of conjugation, nearly 90º with respect to the anthracene plane. Decreasing twist angles toward 45º with increasing pressure serve mainly to enable close face-to-face packing of the anthracenes rather than to extend conjugation by partial planarization.

Quite the same general mode of action holds for the beautifully compact, branched and twisted oligothiazole **15** with a thienyl core.[29] Compared to λ_{em} = 450 nm in solution or polymer matrix, the emission of crystals is red shifted to λ_{em} = 550 nm, compression adds another $\Delta\lambda_{em}$ = +50 nm. Crystal structures suggest once again that changes in packing rather than planarization of the twisted fluorophores account for the found mechanosensitivity.

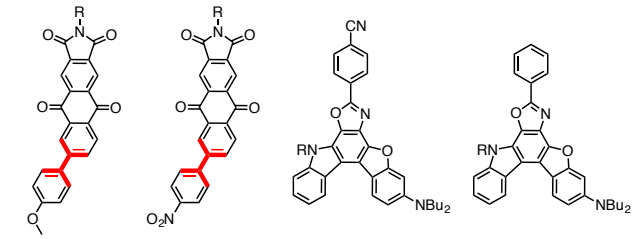

Figure 6. Mechanosensitive materials made from twisted and planar push-pull monomers, with red shifts obtained in response to mechanical stress.

Mechanophore **16** arguably comes closest to a planarizable push-pull probe (Figure 6).[30] An imide acceptor and a methoxy donor are separated by a twist that partially deconjugates the extended aromatic system. Mechanosensitivity, although weak, is demonstrated with a red shift of $\Delta\lambda_{abs}$ = +10 nm to λ_{abs} = 440 nm under pressure. Red-shifted absorption could be consistent with the expected planarization of the push-pull chromophore in the ground state. Loss of mechanosensitivity upon removal of the push-pull system in **17** could be interpreted as experimental support of this interpretation. However, planar push-pull systems show similar or, as with $\Delta\lambda_{abs}$ = +47 nm for **18**, clearly superior mechanosensitivity, and removal of polarization inhibits mechanosensitivity also for planar systems such as **19**.[31] The mechanosensitivtiy of push-pull chromophores is generally explained with the destruction of the antiparallel crystal packing from constructive dipole-dipole interactions.

Taken together, chromophore twisting is popular in mechanosensitive solids to produce loosely packed crystals. Mechanochromism then usually originates from the tightening of this loose packing rather than chromophore planarization. The expectation that mechanochromism from twisted probes in plastic materials upon stretching would be achieved with systems that are better comparable with planarizable push-pull probes in stressed membranes turned out to be incorrect.[32] Their response to stretching invariable originates from the disassembly of supramolecular dimers or higher oligomers. Remarkably, twisted probes for plastic stretching seem to be rare, not to speak of planarizable push-pull probes.

Molecular Rotors

Molecular rotors are somehow the counterparts of planarizable push-pull probes. Whereas the latter are introduced to function as “lobster probes” by planarization of their twisted ground state,[2,3] molecular rotors operate by deplanarization of their first excited state (Figure 7).[33,34] In the most common molecular rotors, this deplanarization of their excited state is thought to occur by twisted intramolecular charge transfer (TICT). Because the decay from TICT states is often

non-radiative, excited-state twisting usually results in quenching rather than in red-shifted emission.

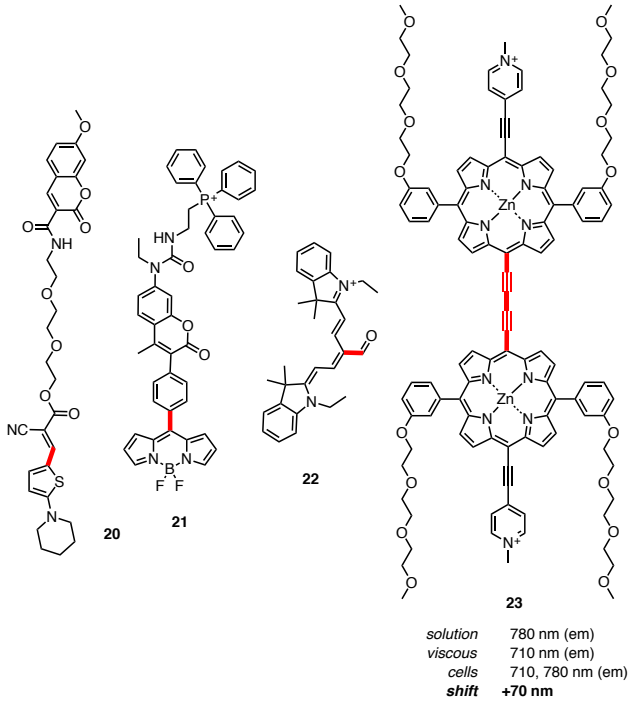

Figure 7. The concept of molecular rotors focuses on deplanarization of their planar excited state, whereas planarizable push-pull probes are conceived to operate by planarization of their twisted ground state. As a result, the former show quenching or red shifts in emission, the latter red shifts in excitation and fluorescence increase.

Molecular rotors are mechanosensitive. For example, excited-state twisting is hindered in confined space or viscous environments. Molecular rotors have thus attracted much attention as fluorescent probes for viscosity. Fluorescence probe **20** contains one of the most popular rotor, that is a push-pull chromophore with a cyanovinyl acceptor and a variable donor, here a most powerful amine (Figure 8).[33] In this rotor, excited-state deplanarization occurs by rotation around the single bond connecting the acceptor with the chromophore. Probe **20** further contains a mechanoinsensitive coumarin fluorophore for internal calibration of the mechanosensitive rotor. This architecture allowed for the λ-ratiometric[35] detection of the viscosity in cells.[36]

Another classical motive with molecular rotors are BODIPY chromophores with central phenyl substituents as in fluorescent probe **21**. Here, rotation around the bond that connects BODIPY and phenyl has been proposed to account for quenching by TICT.[34,37] This rotor has been demonstrated to sense the viscosity in viscous media as well as the nature of lipid bilayer membranes.[34] Namely, fluorescence emission intensity increases from liquid-disordered (L_d), liquid-crystalline or fluid membranes to liquid-ordered (L_o) and solid-ordered (S_o) or crystalline membranes. The detection of changes in fluorescence lifetime rather than emission intensity, particularly with fluorescence lifetime imaging microscopy (FLIM),[38] provides a more quantitative readout that is compatible with the imaging of live cells.

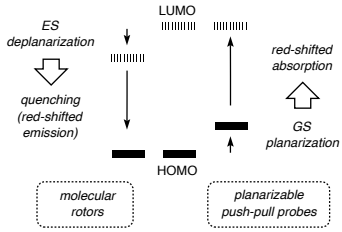

Figure 8. Molecular rotors for viscosity measurements in cells. The twistable bonds in the fluorophores that are thought to account for TICT are highlighted. Probes **20** and **21** further contain mechanoinsensitive fluorophores for ratiometric detection, probe **21** a mitochondrial targeting triaryl phosphonium cation, and probe **23** reports decreasing viscosity with red shifts rather than fluorescence recovery.

Fluorescent probe **21** elegantly combines the latest progress with BODIPY rotors.[37] An insensitive coumarin fluorophore is incorporated for ratiometric detection, and a triaryl phosphonium cation is added to target mitochondria. With this sensor, the

average mitochondrial viscosity could be measured in HeLa cells. High sensitivity toward mitochondria depolarization with ionophores such as monensin or nystatin was observed.

Molecular rotor **22** was also designed for ratiometric imaging and FLIM with cells.[39] An aldehyde was placed in the middle of the cyanine fluorophore. Hindered rotation of this aldehyde in viscous media results in the expected increase in emission intensity and fluorescence lifetime (0.2 to 1.5 ns). Ratiometric detection was possible because only the bathochromic of the two emission maxima of the fluorophore was mechanosensitive. In FLIM images of cells, it was possible to clearly note regional differences in viscosity.

The butadiyne-linked porphyrin dimer **23** exemplifies outstanding conceptual innovation in the field of molecular rotors and beyond (Figure 8).[34,40] Rotation around the single bonds in the diyne bridge have been proposed to account for twisting. Lessons from oligophenylethynyl mechanophores such as **9**-**11** (Figure 4) suggest that deplanarization could occur in ground and excited state, and that planarization in both cases should restore conjugation through the triple bonds and thus result in red shifts in excitation and emission. In viscous media, blue shifts of $\Delta\lambda_{em}$ = +70 nm to λ_{em} = 710 nm were observed. This intriguing response to viscosity was of practical interest for direct ratiometric detection. In HeLa cells, high viscosity was observed, and organelles with different viscosity could be detected with rotor **23**. Constant irradiation of rotor **23** in CHO cells caused cell death. The microviscosity clearly increased during apoptosis induced by photodynamic therapy.

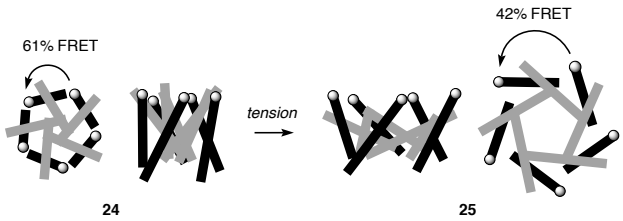

Figure 9. Schematic structures of mechanosensitive channels and their conversion into fluorescent membrane probes. The closed form **24** (left: top, right: side view) is composed of a pentameric helix bundle (grey) that is surrounded by another pentameric bundle (black). Under

tension, this bundle opens into the helical channel **25**. For sensing, fluorophores (shaded circles) are attached to the external termini of the external helices.

Mechanosensitive Channels

Mechanosensitive channels offer an interesting opportunity to bypass the challenge to create mechanosensitive membrane probes from scratch.[41] In lipid bilayer membranes, tension can vary from 10^{-9} N/m (Newtons per meter) to 10^{-3} N/m (lysis tension). The biologically relevant range is 10^{-4}-10^{-3} N/m and expected to be of highest importance in central processes ranging from endocytosis and trafficking to cell division. Mechanosensitive ion channels function as mechanotransducers that generate currents and mediate transmembrane ion transport in response to changes in membrane tension. They are implicated in touching, hearing, muscular coordination and so on, and they prevent cells from lysis in response to osmotic stress.

In closed form, these channels are transmembrane α-helix bundles composed of a pentamer (**24**, Figure 9, grey) that is surrounded by another pentamer (**24**, Figure 9, black). In response to lateral membrane tension, the closed bundles **24** open up like flowers in bloom to afford the open channels **25**. To turn these biological mechanosensors into fluorescent probes, fluorophores have been attached at the external terminus of the α-helices of the outer pentamer. In closed form, the bioengineered probe **24** exhibited 61% FRET (Förster resonance energy transfer). Opening under tension increased the distance between the fluorophores in the mechanosensitive channels **25**. As a result, FRET decreased to 42%. For practical applications, the usefulness of active transmembrane channels as non-invasive mechanosensitive probes remains to be confirmed. From a structural point of view, it is interesting to note that mechanosensitive channels do not operate by planarization of twisted monomers. Like most mechanosensitive organic materials (Figures 5 and 6), mechanosensitive channels operate by changes of the packing of the involved monomers in response to mechanical stimulation (Figure 9).

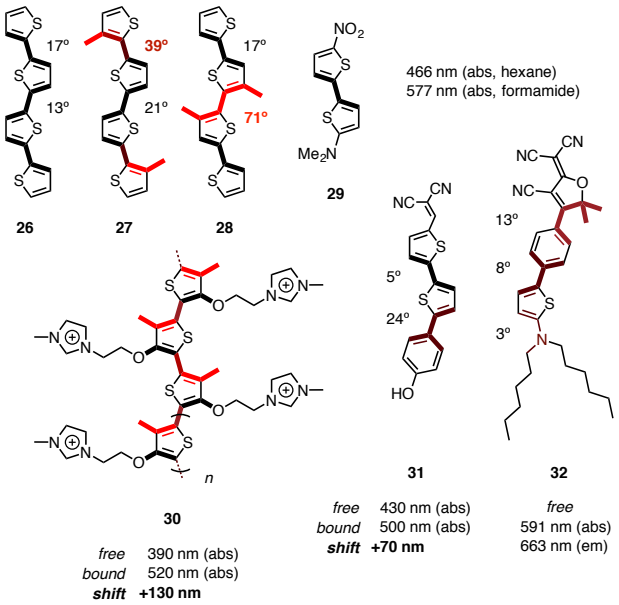

Figure 10. Pertinent examples for the deplanarization and polarization of oligo- and polythiophenes. Selected torsion angles are indicated along the scaffolds (mostly computational), as are red shifts caused by binding to DNA (**30**) and amyloid-β proteins (**31**).

Oligothiophenes

The concept of planarizable push-pull probes was originally explored with oligothiophenes.[2,42] This choice was made because both the deplanarization and the polarization of oligothiophenes as separate processes is very well documented. Oligothiophenes **26**-**28**, for example, have been analyzed in detail as model compounds with zero, one or two methyl groups next to the twistable bond connecting two thiophenes (Figure 10).[43] In their first excited state, they all are planar. In the ground state, the twist between rings without methyl deplanarizers is up to 21° in calculations and 0° in the crystal. The introduction of one deplanarizer gives a 39° twist from *anti* conformation in theory and still full planarization in the crystal. With two proximal methyl groups, twist angles in the crystal can vary from 0° to 30° and 46°. The calculated twist angle with two proximal methyl groups is 71°. Extensive material available on push-pull systems can be traced back to early insights that strong donors and acceptors as in bithiophene **29** generate significant red shifts with

strong solvatochromism.[44] Photoinduced intramolecular charge transfer affords highly polarized excited states that can be stabilized by dipole-dipole interactions with polar solvents.

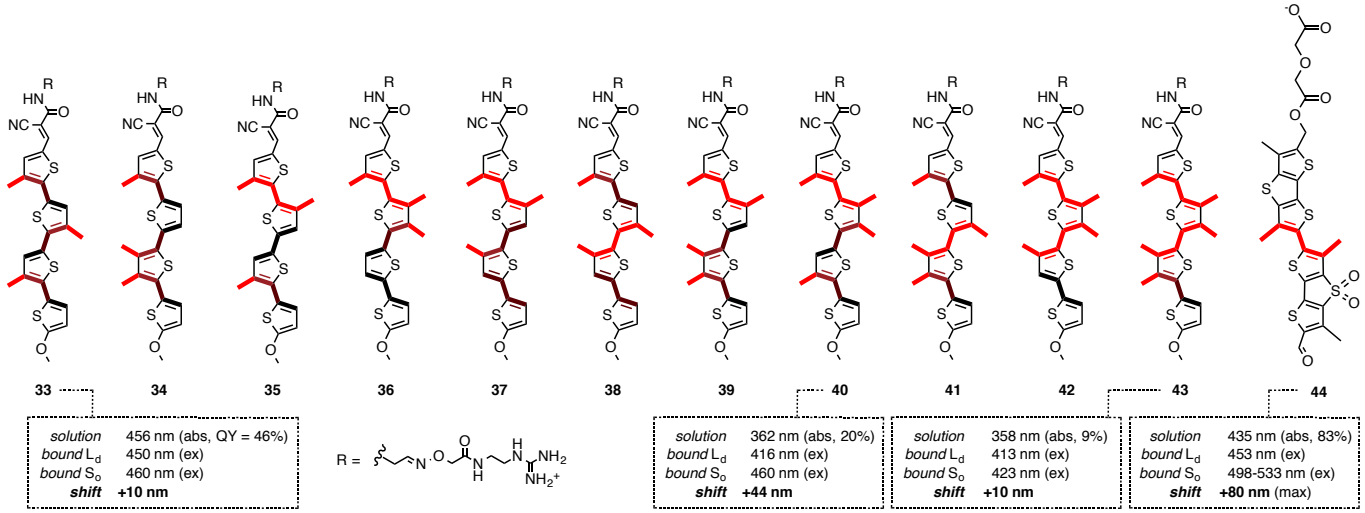

Figure 11. Planarizable push-pull probes, covering the oligothiophene twistome 33-43, and fluorescent flipper 44, conceived to assure high mechanosensitivity and long lifetime. Indicated are selected absorption maxima and quantum yields (QY) in solution, excitation maxima in L_d and S_o membranes, and the red shift obtained to discriminate between the two.

The arguably most impressive example for twisted oligo- or rather polythiophenes as planarizable probes comes from the group of Mario Leclerc at Laval University in Quebec.[45] Polythiophene twisting was explored comprehensively, covering essentially the full “twistome” of the system. These efforts on twistomics were rewarded with probes such as polymer **30**. In this polymer, methyl groups are placed on each thiophene for deplanarization. Proximal alkoxy groups support planarization by attractive S-O interactions. These opposing forces seem to afford best mechanosensitivity. The imidazolium cations are added for multivalent binding to polyanions. Planarization upon ion pairing with single- or double-stranded DNA shifted the absorption maximum by $\Delta\lambda_{abs}$ = +130 nm to λ_{abs} = 520 nm. This red shift in response to the planarization without obvious contributions from aggregation is outstanding. Alternative explanations, although always difficult to exclude fully, are quite unlikely.

The planarizable polythiophene probe **30** is not polarized, i.e., it is not a push-pull chromophore. The combination of planarization and polarization has been considered previously, and thiophene were involved in both cases.[46,47] According to calculations, push-pull probe **31** is planar with respect to the two thienyl rings but twisted by 24° between thienyl and phenyl rings.[46] This computational twist needs to be considered with reservation, experimental and theoretical data for the same in **32** indicate that the thienyl-phenyl twist is negligible (8°).[47] Binding of probe **31** to aggregated amyloid-β proteins (Alzheimer's disease) caused the absorption to shift by $\Delta\lambda_{abs}$ = +70 nm to λ_{abs} = 500 nm. Emission did not shift but showed highly increased intensity. These important changes presumably originate from H aggregation and quenching of the hydrophobic probe in water. Significant contributions from the planarization of the nearly planar probe upon binding to the β-sheets can be excluded. The combination of deplanarization and polarization was also considered explicitly for push-pull fluorophore **32**.[47] However, deplanarization was confirmed theoretically and experimentally to be very weak, and probe characterization did not proceed much beyond the routine collection of spectroscopic data in solution.

Planarizable Push-Pull Probes

As stated in the introduction, the objective with planarizable push-pull probes is to create mechanosensitive membrane probes that change color like lobsters during cooking (Figures 1 and 2). This combination of planarization and polarization is expected to report key characteristics of membranes such as disorder, tension or potential as changes in excitation (Figure 7). This response differs clearly from the changes in emission with established membrane probes such as molecular rotors or solvatochromic push-pull probes.

The concept of planarizable push-pull probes was first elaborated with oligothiophenes.[2] Variation of length, donor and acceptor suggested that quaterthiophenes with methoxy donors and cyanovinyl acceptors would perform best. A charged terminus was introduced to assure delivery to and oriented partitioning into lipid bilayer membranes. To find the perfect twist,

deplanarization was studied comprehensively with three to five methyl deplanarizers placed variably along the scaffold of probes **33**-**43** (Figure 11). Deplanarization was assessed from blue shifts of absorption or excitation maxima in solution, planarization from red shifts in L_d and S_o membranes. Deplanarization increased with increasing number of deplanarizers and increasing number of strong twists with two deplanarizers next to the twistable bond. In solution, the absorption maximum of the least deplanarized probe **33** was at λ_{abs} = 456 nm. The most deplanarized probe **43** absorbed at λ_{abs} = 358 nm. Planarization of the weakly twisted probe **33** from L_d to S_o membranes was with maximal $\Delta\lambda_{ex}$ = +10 nm ineffective simply because there is little there to planarize. Planarization of the overtwisted probe **43** from L_d to S_o membranes was similarly ineffective because planarization is becoming too difficult. Best mechanosensitivity was found at intermediate twist in probe **40**. The change from L_d to S_o membranes was reported with a red shift of $\Delta\lambda_{ex}$ = +44 nm.

With increasing deplanarization, quantum yields dropped from 46% for the undertwisted probe **33** to 9% for the overtwisted probe **43**. The ideal intermediate twist of probe **40** conicided with an intermediate quantum yield of 20% in solution. Decreasing fluorescence with increasing twist was understandable because isolated thienyl monomers are not fluorescent. Planarization in S_o membranes coincided with a strong increase in fluorescence intensity.

Fluorescent Flippers

To create planarizable push-pull probes with higher mechanosensitivity and longer lifetime, the concept of fluorescent flippers was introduced.[3] Fluorescent flippers are bright and big monomers in oligomers that continue to shine when the oligomers are twisted out of conjugation and feel the environment really well. Dithienothiophenes and their S,S-dioxides[48] were considered first as fluorescent flippers.[3] In flipper probe **44**, the two are placed to contribute to the push-pull macrodipole. Further present are an aldehyde acceptor, an admittedly weak alkyl donor, and a negative charge for delivery and orientation. Compared to the best probe **40** at

20%, quantum yields in solution, that is in twisted form, increased to 83% for flipper probe **44**. Planarization from L_d to S_o membranes gave a red shift of up to $\Delta\lambda_{ex}$ = +80 nm in excitation, whereas emission didn't change. Fluorescent lifetimes increased from up to 760 ps for **40** to up to 4.3 ns for flipper **44**. Most important is that the response of planarizable push-pull probes to changes in their environment is independent on their concentration in the membranes. This concentration independence suggested that they really act as isolated push-pull mechanophores that are planarized in response to mechanical stimulation, here the transition from L_d to S_o membranes.

Although further improvable, the spectroscopic properties of flipper probe **44** were sufficient to initiate studies in GUVs and cells. In mixed membranes, the flipper probes **44** distributes equally without strong preferences. As a result, different microdomains could be illuminated with the same probe using different wavelength of excitation (Figure 12). To transcribe the significant changes in excitation to changes in emission, a simple FRET approach has been introduced. In the presence of an additional FRET donor, red shifts of up to +80 nm in excitation generated red shifts of up to +140 nm in emission.

Conclusions

In this concept article, pertinent insights are collected to evaluate the idea to create mechanosensitive fluorescent probes by combining polarization and planarization. It is shown that phenomena similar to the twisting of push-pull chromophores occur quite often in nature. Examples include the chemistry of vision or the pigmentation of lobsters, crabs and plant flowers. However, a closer look inevitably reveals that the involved systems are very complex and the exact contributions of chromophore planarization and polarization are very difficult to extract. Quite the same can be said for mechanochromic materials. They often contain twisted monomers, sometimes also push-pull fluorophores. However, the origin of color change in response to mechanical stimulation is most often seen in changes the packing of the twisted

monomers. Changes in packing also account for the mode of action of mechanosensitive channels. The most impressive impact of planarization on mechanochromism is arguably reported for elegantly twisted phenylethynyl oligomers and polymers as well as very convincing polythiophenes that change color when planarized along oligonucleotides. These polymers are obviously unrelated to push-pull fluorophores and are not polarized at work either.

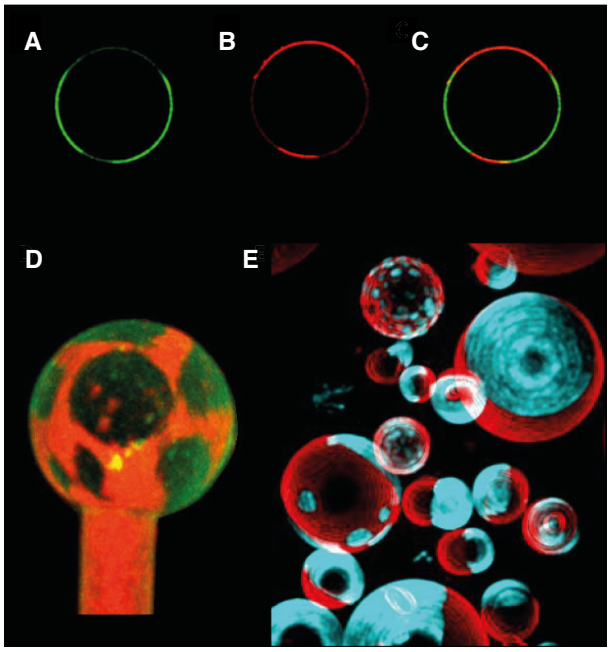

Figure 12. Images of mixed GUVs with L_o and L_d domains labeled with flipper probes **44**. Excitation at short wavelength illuminates L_d phases (λ_{ex} = 480 nm, A), excitation at long wavelength L_o phases (λ_{ex} = 520 nm, B). Merged images show equator region (C), complete GUVs reconstructed from z-scans (immobilized on a micropipette, D), and co-labeled GUVs with ATTO647N in the L_d phase (E, cyan, λ_{ex} = 630 nm). Reproduced from reference [3] with permission, © 2015 American Chemical Society.

Considering the background elaborated in this article, it can be said that the identification of twisted push-pull mechanophores that act as monomers by combining planarization and polarization is quite special. The conclusion is that the likely origin of their mechanochromism will have to be verified and understood first in all details. Once confirmed and validated, the quite unique opportunity to study the planarization of twisted push-pull chromophores in response to

mechanical or, more generally, physical or chemical stimulation can be explored to the fullest. Promising perspectives reach from fundamental studies on mechanosensitive bonds down to the single-molecule level to important questions in biological and materials sciences.

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