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Soft-Mechanochemistry: Mechanochemistry Inspired by Nature

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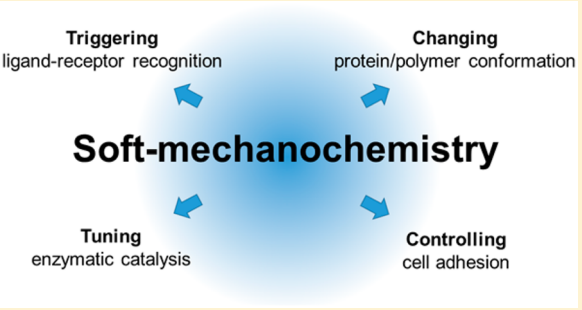
Supporting Information

ABSTRACT: Cells and bacteria use mechanotransduction processes to transform a mechanical force into a chemical/biochemical response. The area of chemistry where chemical reactions are induced by mechanical forces is called mechanochemistry. Over the last few years, chemists developed force-induced reactions affecting covalent bonds in molecules under tension which requires high energy input and/or high intensity forces. In contrast, in nature, mechanotransduction processes take place with forces of much weaker intensity and much less demanding energy. They are mainly based on protein conformational changes or changes in supramacromolecular architectures. Mechanochemistry based on such low-energy-demanding processes and which does not affect chemical bonds can be called soft-mechanochemistry. In this feature article, we first discuss some examples of soft-mechanochemistry processes encountered in nature, in particular, cryptic sites, allowing us to define more precisely the concepts underlying soft-mechanochemistry. A series of examples, developed over the past few years, of chemomechanoresponsive systems based on soft-mechanochemistry principles are given. We describe, in particular, cryptic site surfaces, enzymatically active films whose activity can be modulated by stretching and films where stretching induces changes in their fluorescence properties. Finally, we give our view of the future of soft-mechanochemistry.



I. INTRODUCTION

How can mechanical forces affect or trigger chemical reactions? This question might sound strange to a young chemistry student since mechanical forces are never introduced, or only very rarely, in basic chemistry courses. Yet, even if it was recognized as early as in 1930 by Staudinger[1] that strong shear forces allow breaking covalent bonds in macromolecular chains, it has been only 10 years since researchers have actively tried to design molecules affected by the action of mechanical forces.[2] These targeted reactions concern intramolecular processes in constrained rings and often lead to color changes or luminescence emissions (mechanophores). In agreement with the IUPAC definition, the branch of chemistry where chemical reactions are induced by mechanical forces is called mechanochemistry. One of the pioneers in this field is the group of Jeffrey S. Moore, which introduced a system that has become a benchmark in the domain.[3] It consisted of a material based on polymers containing spiropyran rings in their chains which under stretching transformed into merocyanines (Figure 1a) and thus changed color. Many other systems have been reported since by many different groups, and significant examples are shown in Figure 1.[4−8] Even if it is very elegant and addresses many novel fundamental questions in chemistry,[9,10] this approach requires high-intensity forces and high energy input. These chemical transformations are often triggered by ultrasound[6−8] or the stretching of rubbery materials close to or beyond their rupture point.[4,5] Moreover, these processes are usually irreversible, meaning that when the forces acting on the molecules are removed, the initial states are not recovered.

Mechanochemistry processes also play a great role in nature, for example, in the perception of movement and spatial orientation,[11] touch sensitivity,[12] blood clotting,[13] and more generally in the interactions of cells with their environment. Interestingly, these processes take place at much weaker forces than required to affect chemical bonds. One can therefore wonder how nature manages to transduce weak forces into chemical signals. The basic mechanisms always rely on conformational changes either in entire supramolecular arrangements, as in the case of ion channel openings in cell membranes,[14] or conformational changes of single proteins, as in the case of interactions of cells with their environment.[15] These processes do not affect chemical bonds, are thus much more energy saving, and are often reversible, meaning that when the mechanical forces are withdrawn, the systems recover their initial state. A biomimetic strategy can be developed,

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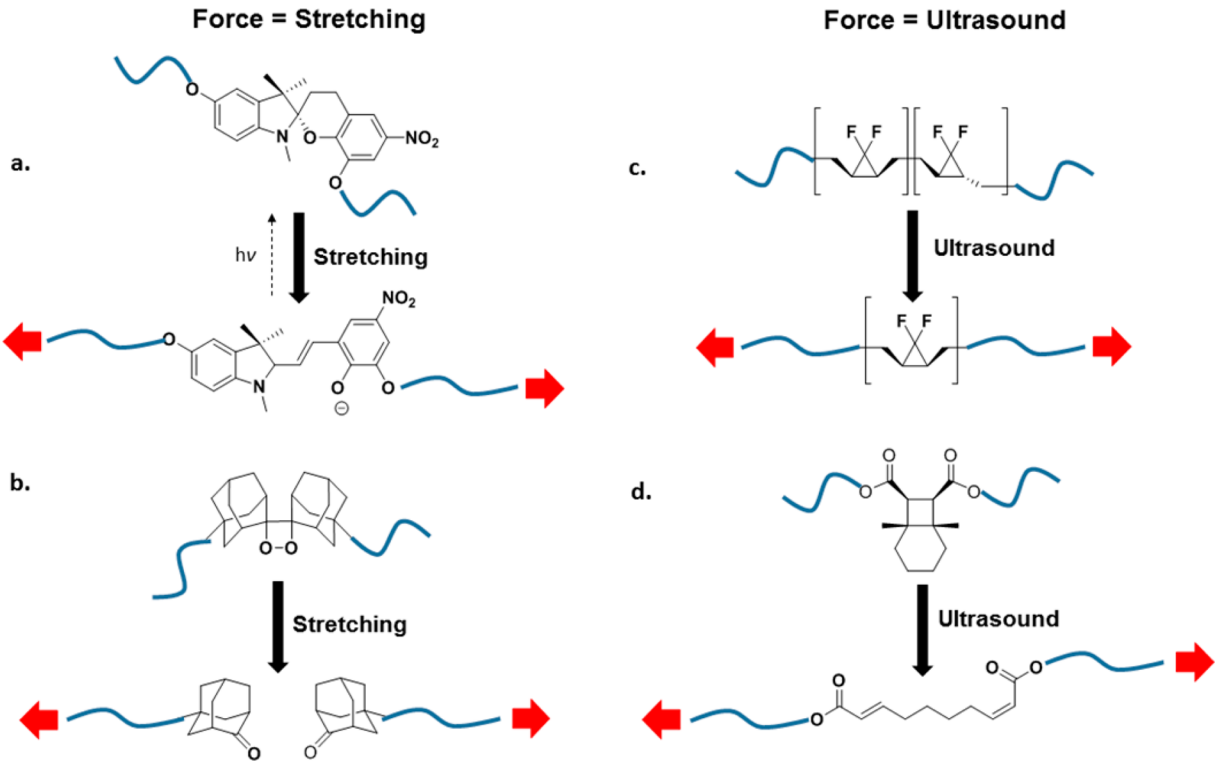

Figure 1. (a) Uniaxial stretching of the material induces a force on the constrained spiropyran ring, leading to the formation of merocyanine groups.[3] (b) The stretching of the 1,2-dioxetane ring included in polymers leads to the breaking of chains through adamantanones formation.[10] (c) Fluorinated polycyclopropane with trans and cis configurations is rearranged under ultrasound to provide a polymer exhibiting a cis configuration exclusively.[6] (d) Ultrasound allows the ring opening of cyclobutane included in polymer chains.[7]

namely, to build macromolecular systems (either of single macromolecules or complex systems composed of several macromolecules), which under the influence of mechanical forces change the conformation in such a way that they induce chemical processes. By chemical processes, we mean the specific interaction between ligands and receptors or the catalysis of a chemical transformation. In contrast to conventional mechanochemistry, this approach can be called soft-mechanochemistry, a field that is still in its infancy (Figure 2).

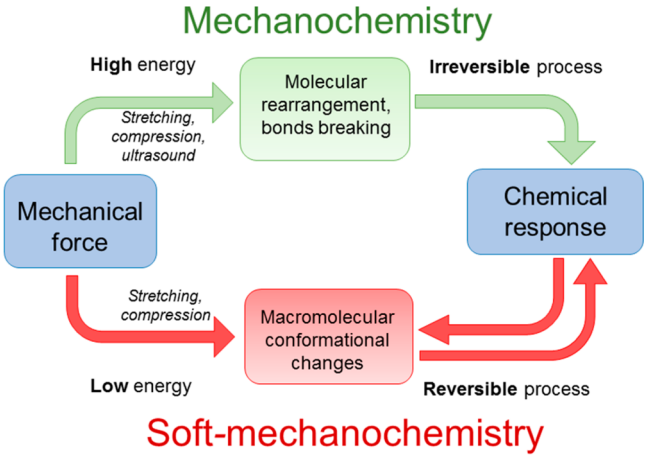

Figure 2. Schematic representation illustrating the concept of soft-mechanochemistry compared to classical mechanochemistry.

In this feature article, we will introduce some soft-mechanochemistry principles based on examples found in nature: mechanotransduction processes mediated through protein conformational changes. The emerging key concept of cryptic sites will be defined. We will then show how soft-mechanochemistry principles can be used to design original and efficient mechanoresponsive surfaces. These examples will mainly be based on our own contributions. It must be mentioned that in parallel to our own investigations, another group has also created chemical systems displaying mechanical responsiveness through conformational changes. Using Langmuir−Blodgett techniques, Ariga's group has demonstrated that mechanical compression on soft monolayers can induce the conformational change of pyranose rings[9] and binaphtyl derivatives.[16] Finally, from our own experience in this field, a discussion of the future of the soft-mechanochemistry field will conclude this feature article

II. SOME EXAMPLES OF MECHANOCHEMISTRY PROCESSES IN NATURE

One major way used by nature to transduce mechanical signals into chemical responses is through protein conformational changes. We will present here two representative examples of force-induced protein conformational changes that lead to a chemical response. These examples are by far not exhaustive. Aimed to illustrate one strategy used by nature to perform mechanotransduction processes, they can inspire the design of mechanoresponsive materials.

II.A. Talin: A Protein Involved in Cell Adhesion Processes. Cells interact with their extracellular matrix through membrane integrin clusters. These clusters are linked to the actin cytoskeleton through proteins that regulate the adhesion process. Key players in this connection and regulation are talin molecules, proteins that directly interact both with integrins, actin, and vinculin.[17] In order to adapt to their environment, cells apply forces on the extracellular matrix through their actin fibers.

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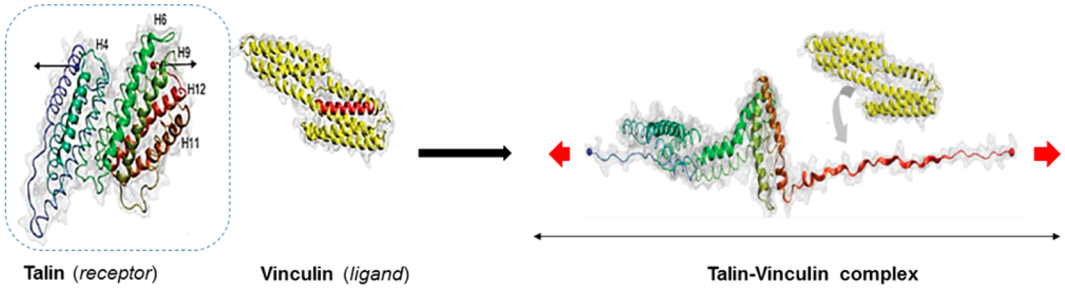

Figure 3. Representative example of a natural cryptic site protein that exhibits buried sites under the action of a mechanical force: when talin is mechanically stretched in a uniaxial way, buried adhesion sites become available to allow the recognition process with vinculin, its natural endogenous ligand. From ref 19 (adapted with permission from AAAS).

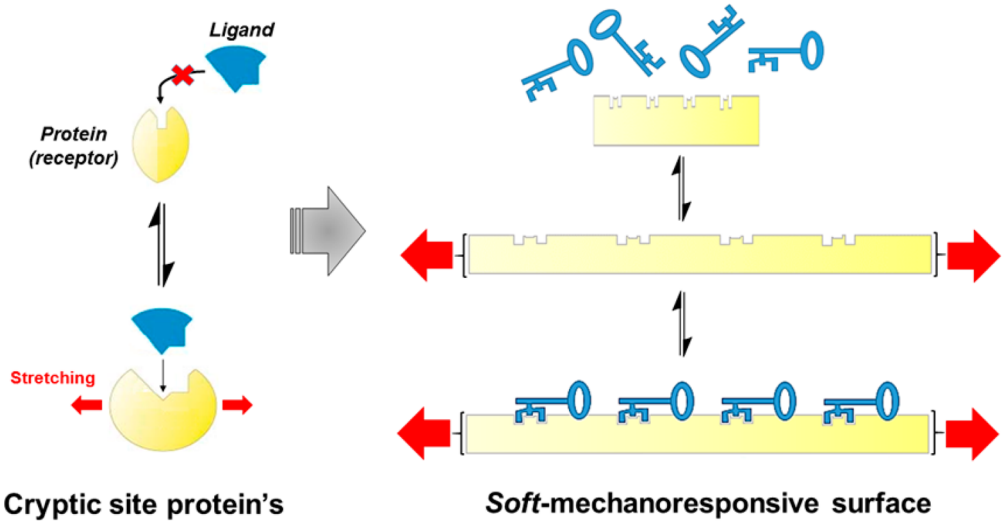

Figure 4. Schematic representation of new smart materials inspired by mechanisms of cryptic site proteins.

These forces are transmitted from the actin fibers to the extracellular matrix through the talin molecules which change conformation under stretching. This conformational change induces the binding of vinculin onto talin. The interaction of vinculin with talin through the stretch-induced talin conformational change reinforces the talin−actin linkage.[18] More precisely, talin is a protein composed of a head and a tail. The globular head of talin contains a domain that binds β-integrin cytodomains, and the talin tail is composed of 62 helices folded into 13 helical domains that contain multiple vinculin binding sites. These vinculin binding sites are defined by six turns of an α-helix and are buried in a helical domain because of hydrophobic interactions. When a talin molecule is stretched due to mechanical forces, some helical domains unravel, exhibiting the vinculin binding site (Figure 3). Such sites are called cryptic sites: sites which are buried at rest and become exhibited under stretching. The exhibition of these sites allows vinculin to interact with actin and to change its conformation in such a way that it surrounds the binding site, burying 50% of its water-accessible area.

II.B. von Willebrand Factor: A Protein Responsible for Blood Clotting. The von Willebrand factor (vWF)[13] is a fascinating biomacromolecule that is the first actor entering in action after vascular damage to induce rapid blood clotting. It is a large multimeric glycoprotein whose number of monomer units, up to 200-mers, adapts to maintain homeostasis. Each monomer interacts with a large variety of compounds: collagens, platelets, proteases, and clotting factors. The different interaction sites are distributed over different domains within a monomer. In the absence of vessel injury, the vWF is in a quiescent state with a coil conformation. As soon as a vessel injury takes place, collagen from the vessel becomes exposed, leading to immediate interaction with vWF molecules through apparent interaction sites. Then, the blood hydrodynamic flow generates strong stretching forces on the attached vWF molecule, leading to conformational changes and to the exhibition of different sites. Some of the exhibited sites induce strong interactions with platelet GPIbα receptors which participate in the clot formation whereas other sites can be cleaved enzymatically and participate in clot resorption. vWF is a molecule which can be considered to be a condensate of soft-mechanochemistry principles: force-induced cryptic site exhibition and conformational site regulation. These two examples are not exceptions in nature. Indeed, many other well-characterized mechanoresponsive biological systems involving proteins, fibronectin,[15] or tenascin-C,[20] for example, might complete this list.

Thus, nature makes widespread use of conformational changes, leading to site exhibition or affinity modifications of specific sites to regulate chemical processes. Such processes have inspired the design of chemomechanoresponsive systems[21,22] with mechanotransduction processes requiring far less energy input than for processes involving bond breakage (Figure 4). These are the main principles of soft-mechanochemistry.

III. CHEMOMECHANORESPONSIVE MATERIALS BASED ON SOFT-MECHANOCHEMISTRY PRINCIPLES

In order to get a better idea of how the mechanical stress is applied to the systems reported below, some of the devices

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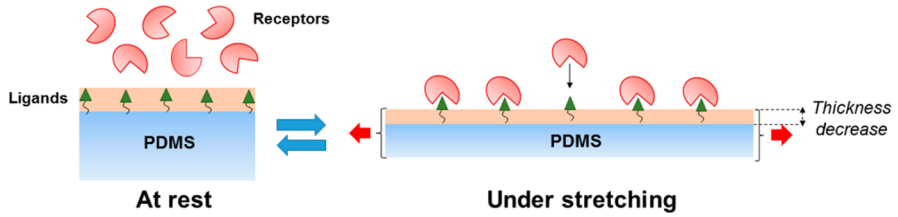

Figure 5. Schematic representation of a mechanoresponsive material exhibiting cryptic sites under stretching: a surface of poly(dimethylsiloxane) (PDMS), an elastomeric material, is modified by a polymeric coating to provide hidden sites at rest that are exhibited under stretching.

used to perform the reported results are supplied as Supporting Information.

III.A. Cryptic Site Systems. As described previously, site exhibition is one of the strategies used by nature to induce biological responses through the application of mechanical forces. In order to design an original class of smart materials using this cryptic site strategy, one can proceed quite generally as represented schematically in Figure 5.

The surface of a selected material has to be modified in order to provide hidden sites (cryptic sites) at rest and site exhibition when the material is stretched. The material has to be an elastomer to expect a reversible effect. The targeted surface properties could be obtained through a polymer coating. The first artificial system mimicking the natural cryptic site strategy was based on polyelectrolyte multilayer films applied on a poly(dimethylsiloxane) (PDMS) sheet, used as an elastomeric material.[23] Polyelectrolyte multilayers are films obtained by the alternate deposition of polyanions and polycations.[24] They are a convenient tool to functionalize all kinds of surfaces, including silicone sheets, in a well-controlled manner.[25,26] An anchoring precursor multilayer made of two poly(allylamine)/poly(styrenesulfonate) bilayers $(PAH/PSS)_2$ was first deposited onto a silicone sheet to avoid any influence coming from the nature of the surface. This precursor film was then covered with a (PAH/PAA-biotin) bilayer where PAA-biotin represents poly(acrylic acid) chains functionalized at 5% with biotin moieties. Biotin is the natural ligand of the protein called streptavidin. The biotin/streptavidin interaction is among the strongest noncovalent interactions known ($K_d = 10^{-14}$). It is considered to be an ideal model of ligand/receptor systems. The biotin groups were embedded under two (PAH/PAA-PC) bilayers, where PAA-PC represents poly(acrylic acid) functionalized at 5% with phosphorylcholine (PC) moieties (Figure 6a). PC is known to render surface antifouling when used in multilayer films.[26] When a $(PAH/PSS)_2$-PAH/PAA-biotin/$(PAH/PAA\text{-}PC)_2$ film was brought into contact with streptavidin (labeled with fluorescein thioisocyanate (FITC)), no adsorption onto the surface was observed, indicating the shielding of biotin by the PC protecting groups (Figure 6b). Stretching of up to 50% of the initial length of the modified PDMS led to the recognition of streptavidin by biotin groups. The amount of streptavidin deposited on the surface increased linearly with the degree of stretching (Figure 6c). In the absence of biotin inside the multilayer, no streptavidin deposition was observed. Arginine-glycine-aspartic acid (RGD), a cell adhesion peptide, was also grafted onto the poly(acrylic acid) chains instead of biotin, and the same type of multilayer, i.e., $(PAH/PSS)_2$-PAH/PAA-RGD/$(PAH/PAA\text{-}PC)_2$, was built. No cell adhesion was observed at rest on this surface whereas a regular increase in cell adhesion (and viability) took place when the film was stretched up to 50%. Considering both ligand−receptor couples used, biotin−streptavidin and RGD−cells, the stretching of modified PDMS with adequate multilayer films led to an exhibition of buried sites (biotin groups or RGD sequence) which can be considered to be cryptic sites by analogy to the systems developed by nature.

Thus, the specific supramolecular recognition between a ligand grafted on a surface and its receptor, free in solution, can be tuned by the mechanical force applied to the material. However, the stretch-induced interaction of streptavidin and the adhesion of cells on the surface were not reversible: when returning to the nonstretched state, the streptavidin or the cells did not detach from the surface. When an RGD (respectively biotin)-based system was first stretched and then returned to the nonstretched state before coming into contact with the cells (streptavidin), cell adhesion (streptavidin interaction) was observed, indicating that stretching irreversibly modified these systems. This irreversibility may be related to a reorganization of polyelectrolyte chains in the multilayer under stretching.

In order to circumvent the irreversibility of the exhibition process, we designed another cryptic site system where both ligands, biotin and RGD peptide sequence, were shielded by an alternative polymer coating strategy: poly(ethylene oxide) chains (PEO) that were covalently grafted onto silicone sheets[27] to design brushes all along the surface (Figure 7). A PDMS sheet was first functionalized through plasma polymerization of maleic anhydride, providing a high surface density of carboxylic groups. Then, the so-activated PDMS was stretched and NH_2-PEO chains, NH_2-biotin, or NH_2-RGD moieties were grafted onto the surface using carbodiimide chemistry. When returning to the nonstretched state, the larger PEG chains hid the ligands (biotin or RGD) and prevented them from coming into contact with macromolecular receptors. It appeared that a 45 EO chain was an optimal length for shielding. Shorter and longer EO chains did not prevent streptavidin interactions in the absence of stretching, a mandatory property for the system to work efficiently. When stretching the PDMS sheet, the PEG density decreased, allowing some of the ligands to become accessible to their receptors. It has been found that the number of streptavidin molecules interacting with the surface increased with the degree of stretching. These results were corroborated by AFM force measurements. Using AFM tips with grafted streptavidin, it was found that by stretching a surface with biotin cryptic sites the force distribution shifted toward higher rupture forces. By stretching, an increasing number of sites thus became accessible to their receptors. Interestingly, this system based on PEO appeared to be fully reversible, in contrast to that based on polyelectrolyte multilayers. This result was surprising because biotin with streptavidin forms the strongest noncovalent bond known in biology. The disruption of this bond when returning to the nonstretched state was hypothesized to be due to a streptavidin conformational change induced by lateral pressure exerted by the PEG chains.

In a second part of the study, biotin was replaced by RGD, and cell adhesion tests were performed with human osteoprogenitor cells. When the cells were seeded on the surface in the

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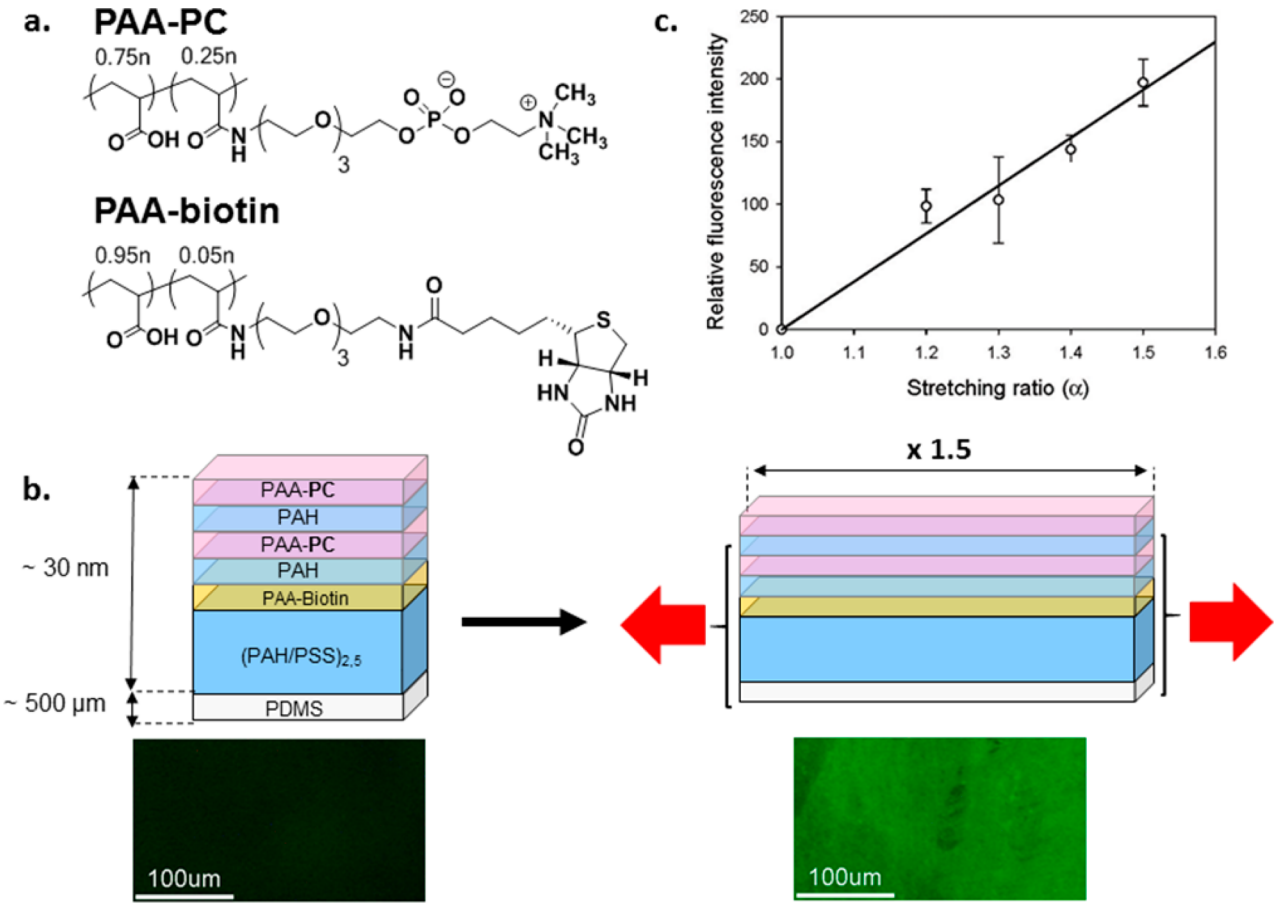

Figure 6. (a) Chemical structures of PAA-biotin and PAA-PC used to build a mechanoresponsive thin film. (b) Schematic representation of the multilayer film architecture adsorbed on PDMS at rest (left) and in the stretched state (right). Under both representations are shown typical images of the film brought into contact with streptavidin labeled with fluorescein isothiocyanate (streptavidin^{FLUO}). (c) Evolution of the fluorescence intensity measured from streptavidin^{FLUO} adsorbed on the multilayer film at different stretching ratios α. The stretching ratio α is defined as the ratio of the lengths of the PDMS sheet after and before stretching. The data correspond to means ± standard deviations from three experiments. (Adapted with permission from ref 23. Copyright 2012 American Chemical Society.)

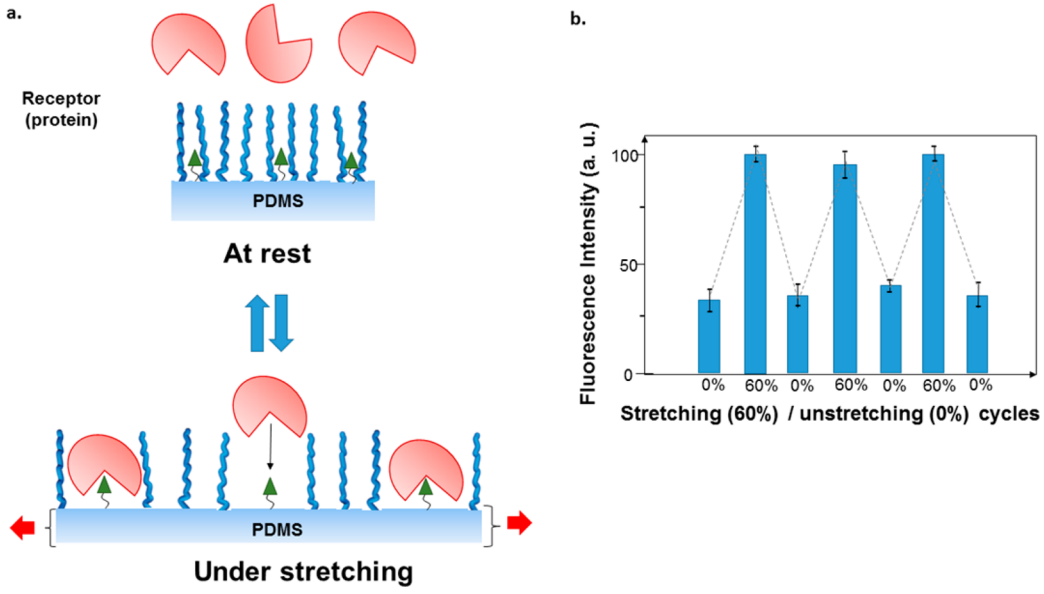

Figure 7. (a) Schematic representation of a reversible cryptic site surface through PEO brushes. (b) Evolution of the fluorescence intensity measured on the PDMS surface and displaying biotin moieties covered by PEO chains after contact with streptavidin^{FITC} and rinsing. (Adapted with permission from ref 27. Copyright 2012 American Chemical Society.)

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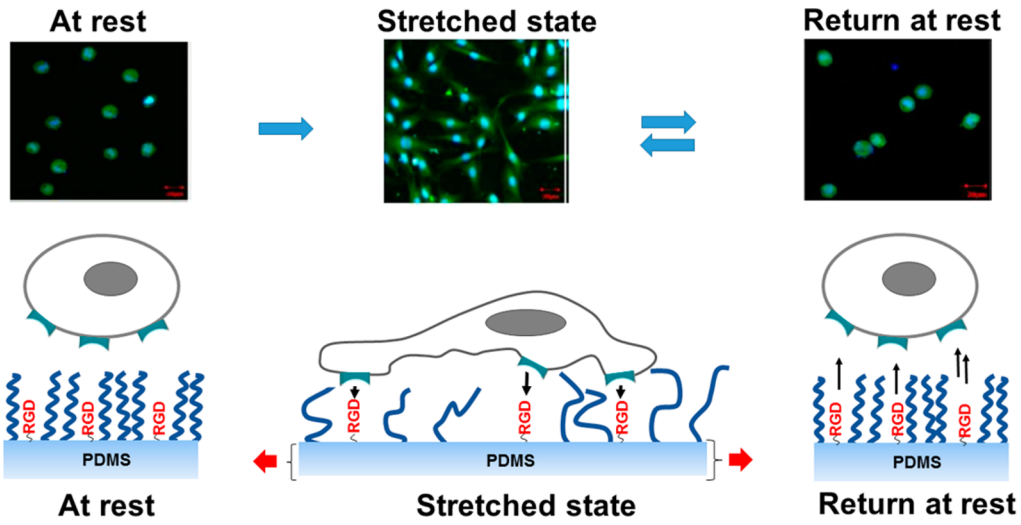

Figure 8. Osteoprogenitor cells seeded on a cryptic site PDMS sheet. (Left) Cells in contact with the PDMS at rest do not adhere and thus retain a typical round shape. (Middle) Cells are seeded on the stretched PDMS. Cells firmly adhere on the surface, indicating the accessibility of the RGD peptides to the integrins (adhesion proteins). (Right) Finally, the PDMS sheet is brought back to the nonstretched state. Cells become round-shaped after 10 min and remain in this state even after 4 h. Sizes are not to scale. (Adapted with permission from ref 27. Copyright 2013 American Chemical Society.)

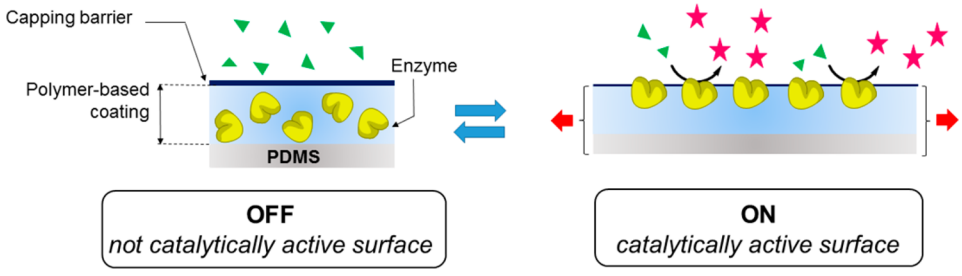

Figure 9. General concept developed by our group to design a mechanoresponsive catalytic surface inspired by the cryptic site strategy described previously. An enzyme is used as the catalyst.

nonstretched state, they remained in a round and nonadherent state (Figure 8). After stretching the PDMS to 60% in the presence of cells for 4 h, cells were spread over the surface and displayed focal adhesions. Cells remained firmly anchored on the surface even if it was rinsed with a buffer solution. This indicates the formation of cellular adhesion probably through specific RGD links. When such a system was brought back to the nonstretched state, focal adhesions disappeared and cells returned to the round nonadhesive state. When such a surface was rinsed, all of the cells were removed from the PDMS sheet, indicating that they lost their adhesion. This again shows that the RGD peptides behave as cryptic sites and that the system is fully reversible.

Cell detachment appears as one of the greatest challenges for using cells after cell culturing.[28] Cell detachment by burying cryptic sites represents a new alternative to already used methods such as the use of thermoresponsive, pH-responsive, photoresponsive substrates. It does not require a change in the chemical nature of the cell-surrounding medium and allows a reuse of the substrate. The next step along this line is to investigate if the technique can also be used to detach cultured cell sheets, which would open new avenues in tissue engineering.

III.B. Cryptic Enzyme Systems. In the cryptic site surface strategies described in the previous part, the specific recognition process between a ligand displayed on a surface and its receptor was controlled by stretching a material. The ligand was qualified as cryptic because it is buried in a polymer-based coating and because it became accessible by stretching the PDMS material. This kind of approach could also be highly interesting for tuning the activity of a catalyst confined on a surface by mechanical stretching. In particular, by controlling the accessibility of the substrate diffusing in the environment to the catalyst, it can be possible to design ON/OFF catalytic mechanoresponsive surfaces (Figure 9).

This approach has been developed by using an enzymatic reaction and two strata of polyelectrolyte multilayers sequentially deposited onto a PDMS sheet.[29] The first deposited film was a poly(L-lysine)/hyaluronic acid multilayer (PLL/HA), an exponentially growing film (i.e., its thickness increases exponentially with the number of deposition steps) that behaves as a viscous liquid. An enzyme, alkaline phosphatase, was embedded in this film simply by diffusion. These PLL/HA multilayers have been shown to maintain the enzymes in an active state after embedding. Then, the film was capped with a linearly growing multilayer built from a poly(diallyldimethylammonium)/poly(styrenesulfonate) (PDADMA/PSS) multilayer, which is known to be more compact. The (PLL/HA) multilayer played the role of a reservoir of enzymes and the (PDADMA/PSS) multilayer, constituted of 6−10 (PDADMA/PSS) bilayers, acted as an efficient barrier to avoid contact between the enzyme and the substrate in the supernatant. Fluorescein diphosphate (FDP) was used as a substrate for alkaline phosphatase. In the nonstretched state, when such a film

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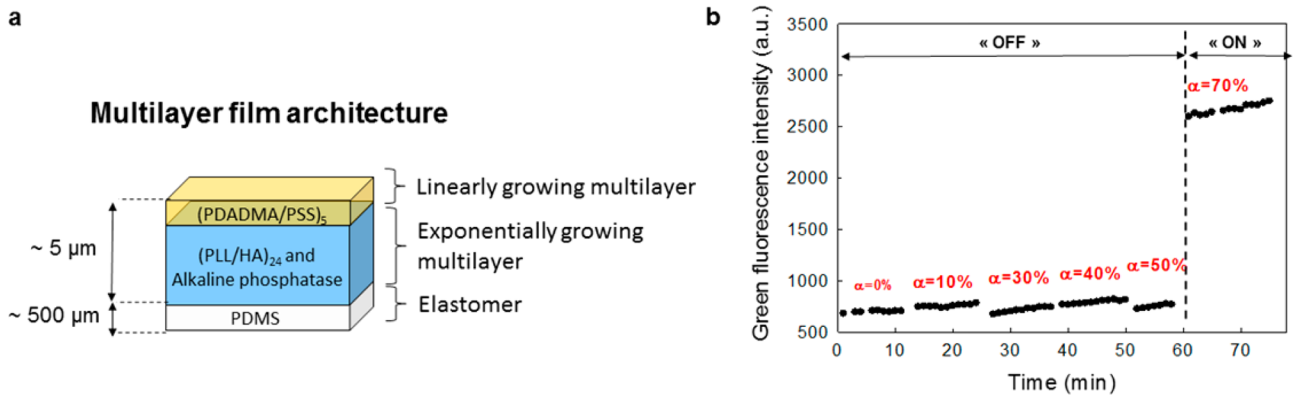

Figure 10. (a) Schematic representation of the multilayer film architecture used to design catalytically active surfaces controlled by a mechanical stretch. Alkaline phosphatase, used as the catalyst, is buried (cryptic) in the exponentially growing multilayer of the film. (b) Evolution of green fluorescence intensity (a.u.) produced according to the degree of stretching: when the PDMS so-modified is stretched at 10, 30, 40, and 50% (α), the transformation of fluorescein diphosphate (FDP) to fluorescein is switch OFF. At 70%, the fluorescence measured in solution strongly increases because of fluorescein production, meaning that the alkaline phosphatase is now able to gain access to the substrate FDP. The catalytic process is thus switch on. Reproduced from ref 29 with permission from Nature Publishing Group.

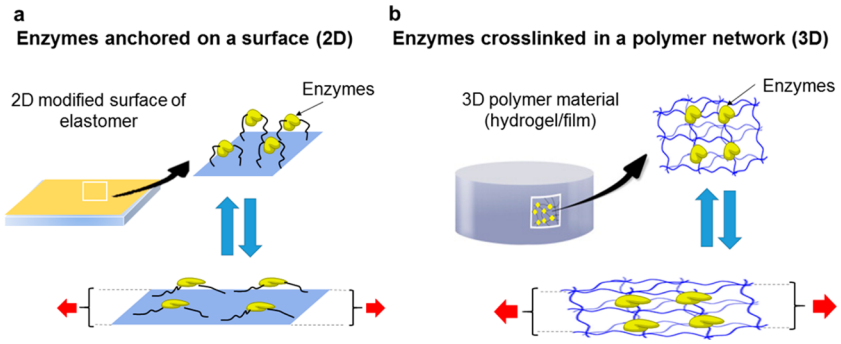

Figure 11. (a) Surface modification (2D) of an elastomer allowing the covalent grafting of enzymes through polymer linkers. When the material is stretched, a mechanical force is directly applied to the structure of the biomacromolecule. (b) Polymer network (3D) made from enzymes used as cross-linking points. When the material is stretched, the deformation of the network induces stresses on the enzymes, which change their conformation and thus their activity.

was brought into contact with a solution containing FDP, no reaction took place.

As soon as the film was stretched up to 70% of its initial length, the enzymatic reaction occurred spontaneously, mainly localized at the interface, leading to the production of fluorescein (Figure 10). When the system was returned to the nonstretched state, the enzymatic activity of the film decreased drastically. This result was interpreted as the stretch-induced exhibition of cryptic enzymes embedded in the highly hydrated film through the capping layer. The exhibition process can be explained by a structural and conformational change of the capping polyelectrolytes constituting the linearly growing film induced by stretching. Moreover, it appeared fairly reversible, which might be due to the fact that when returning to the nonstretched state the PDADMA/PSS multilayer again increases in thickness, preventing the enzymes from being accessible from the solution. Although the tuning of a catalytic reaction by using ultrasound has been reported,[30] this system constituted the first example of a material able to exhibit cryptic catalysts when stretched, switching on a chemical process in a reversible way.

III.C. Modulation of the Enzymatic Activity by Stretch-Induced Enzyme Conformational Changes. Enzymes are efficient catalysts whose activities result from the adequate conformation of the active sites with respect to their substrates. The precise positioning of the different amino acids of the enzymes through their secondary, ternary, and eventually quaternary structures allows specific positioning of the substrate and thus a decrease in the activation energy of the catalyzed reaction. Using this paradigm, one can anticipate that modifying the conformation of an enzyme should result in a change in the molecular positioning of chemical groups involved in its active site, thus leading to a variation (usually a loss) of its enzymatic activity. This can be done either by directly grafting enzymes covalently onto an elastomeric material or by cross-linking enzymes that are embedded in macromolecular reticulated polymer films/hydrogels (Figure 11a,b). By stretching the surface, one can expect that the applied macroscopic force will be transduced locally to the enzyme and will deform it such as to modify the environment in its catalytic pocket.

In 1977, Klibanov et al. were the first to test this idea by covalently attaching enzymes, chymotrypsin and trypsin[31] and later myosin[32] that possesses ATPase activity, onto nylon fibers. By stretching the fibers, they observed a strong reduction of the enzymatic activity. This reduction was reversible. They hypothesized that this behavior can be explained by the "deformation of the enzymes induced by fiber stretching". This observation was confirmed by Ishimori et al. in 1981, who immobilized glucose oxidase on porous polyvinyl chloride membranes.[33] By stretching the membrane, they observed a decrease in the catalytic activity of up to 70%, a process that

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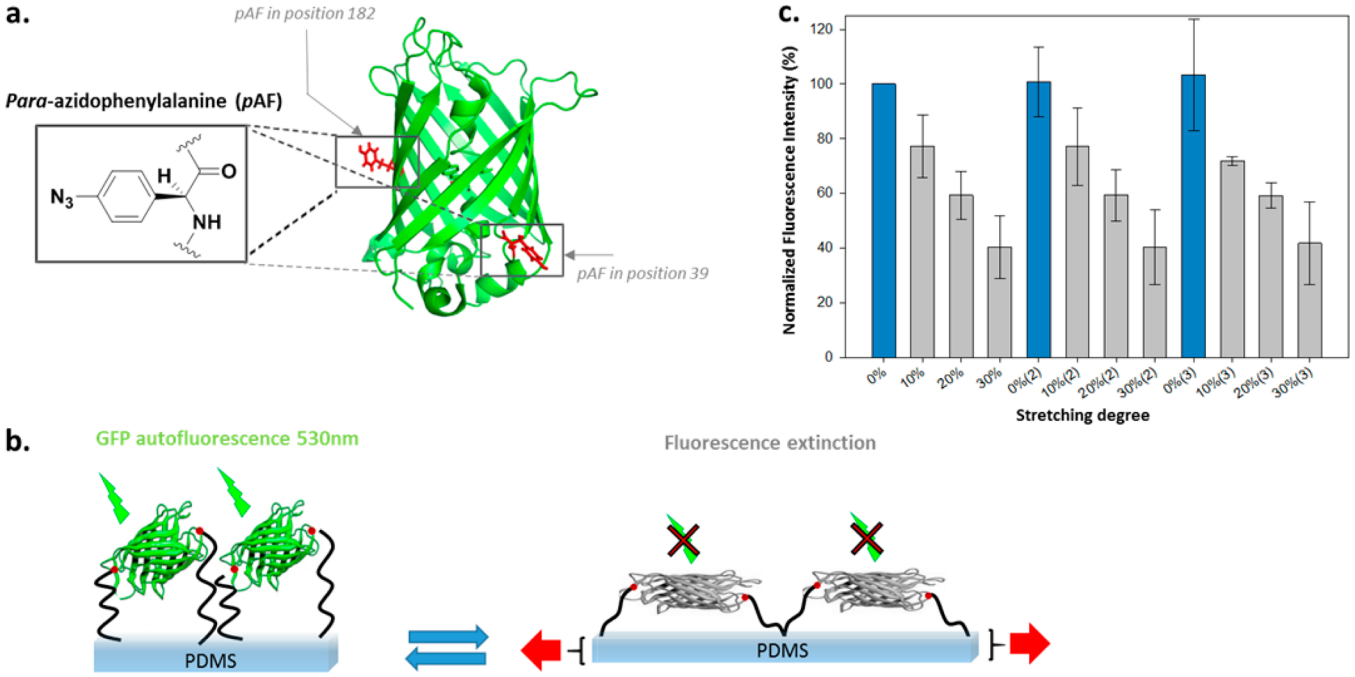

Figure 12. (a) GFP modified genetically at two opposite positions of the β-barrel with a unnatural amino acid bearing a *para*-azidophenyl group (*p*AF). (b) Schematic representation of the GFP covalently linked through POE linkers onto a modified PDMS sheet at rest and stretched. (c) Evolution of the normalized fluorescence of the GFP-modified PDMS as a function of the degree of stretching during three stretching−unstretching cycles. Reproduced from ref 34 with permission from The Royal Society of Chemistry.

appeared in addition quite reversible. Yet, neither Klibanov nor Ishimori proved that the activity decrease was due to a conformational change in the enzymes. This point is a major question in the design and development of mechanoresponsive materials based on biomacromolecules.

In 2015, in order to prove that stretching a material with grafted proteins indeed alters their conformation, we used green fluorescent proteins (GFPs) that were genetically modified at two opposite positions of their three-dimensional structure so as to allow their covalent anchoring onto a PDMS sheet through PEO linkers (Figure 12a,b).[34]

GFP is a protein known to exhibit strong autofluorescence, a feature highly sensitive to conformational changes of its three-dimensional structure.[35] It thus constituted an ideal model for testing the possibility to induce conformational changes by stretching. It must be noted that through single-molecule experiments realized by atomic force microscopy (AFM), Kodama et al. have proved that both stretching and compressing GFP molecules lead to a fluorescence decrease, and they determined that the conformational change was only partially reversible.[36]

In our case, when stretched up to 30%, the fluorescence intensity of the surface decreased and this decrease was linearly proportional to the degree of stretching imposed on the PDMS. Interestingly, the process was fully reversible: when the material was returned at rest, the fluorescence intensity was equivalent to the one measured before stretching. Furthermore, the stretching−unstretching cycle of the material could be repeated at least three times without any loss of fluorescence at rest. This study demonstrated unambiguously that the application of a macroscopic mechanical force on the surface, i.e., stretching, induces the change in the conformation of a biomacromolecule which is reversible for a degree of stretching of at least 30%. It highlights the fact that a macromolecular force can be transduced through polymer chains up to the protein and can modify its three-dimensional structure in a reversible way. It must be noted herein that in 2014 Bielawski and collaborators showed that the incorporation of modified GFP into polymeric material provides a mechanosensitive material where high compression leads to an irreversible decrease in the fluorescence emission of GFP.[37]

In 2015, we designed a mechanoresponsive enzymatic film based on the strategy presented in Figure 11b.[38] We used polyelectrolyte multilayers constituted of HA and PLL-S-TP (poly-L-lysine modified by thiopyridyl groups with a grafting ratio of 27%) deposited on PDMS sheets. This polyanion/polycation pair led, after 24 polyanion/polycation deposition steps, to roughly 5-μm-thick hydrated films. These films were first covalently cross-linked through well-known carbodiimide chemistry. Then, they were brought into contact with enzymes, functionalized by maleimide groups, in the presence of a reductive agent, tris(2-carboxyethyl)phosphine hydrochloride) (TCEP). These maleimide-modified enzymes diffused in the cross-linked HA/PLL-S-TP films where the thiopyridyl moieties were deprotected by TCEP. This allowed a rapid thiol−ene click reaction between the thiol groups and the maleimide moieties and thus the covalent cross-linking of the enzymes onto the HA/PLL matrix.

We used β-galactosidase (Figure 13) as the enzyme and fluorescein di(β-galactopyranoside) as the substrate. β-Galactosidase was selected because it is a tetrameric enzyme having active sites located at the edge of two adjacent subunits. The four subunits are linked through noncovalent interactions. The conformation of this enzyme thus appeared to be particularly sensitive to stretching. By stretching the film up to 30%, we observed a decrease in the enzymatic activity of the film. Interestingly, the change in activity was quite (but not fully) reversible when performing stretching−unstretching cycles (Figure 13b).

III.D. Stretch-Induced Polypeptide Secondary Structural Changes. Soft-mechanochemistry relies on stretch-induced conformational changes and makes use of such changes

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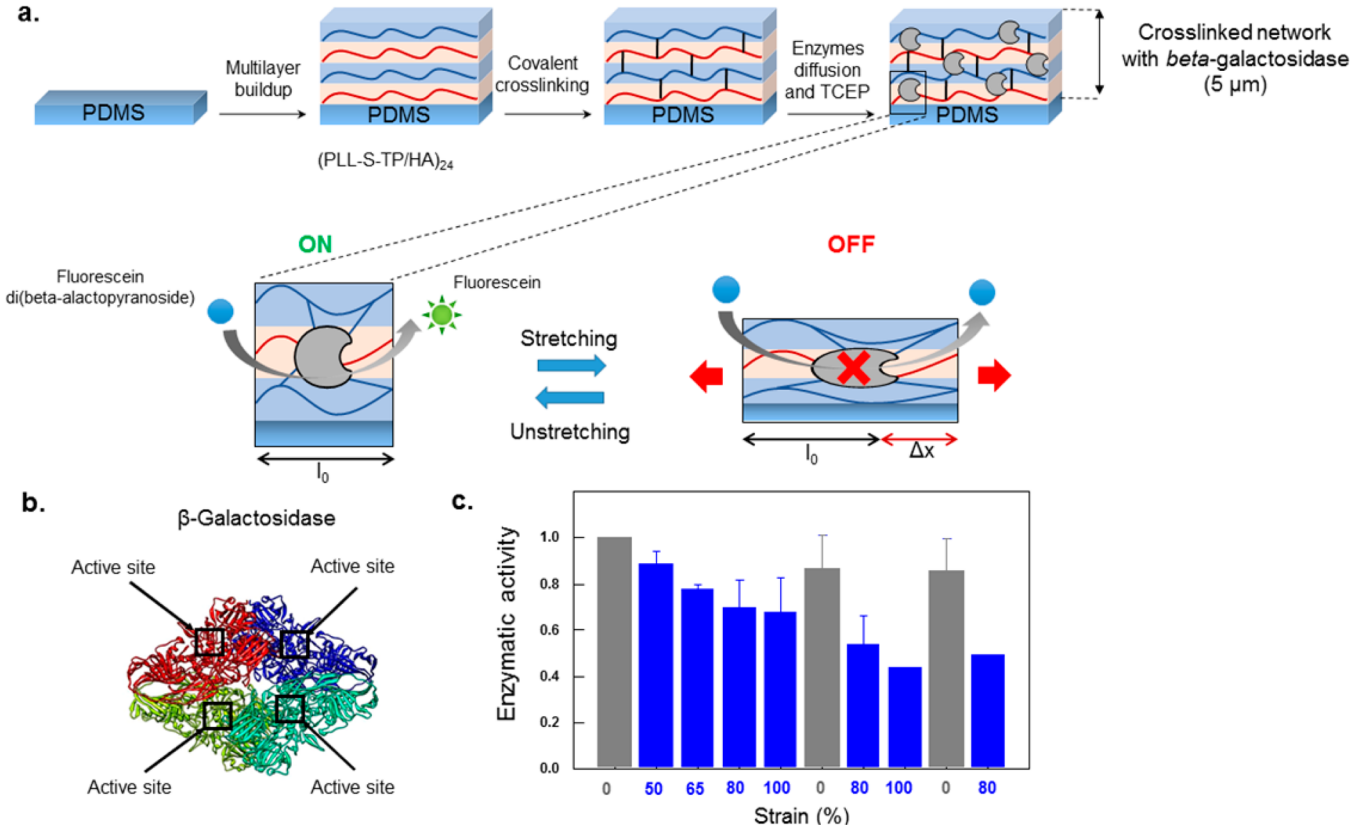

Figure 13. (a) Synthesis pathway for preparing the multilayer exhibiting mechanosensitivity through the conformational change in an enzyme cross-linked into the film. (b) View of β-galactosidase by coloring each subunit. Black arrows show the localization of the four active sites of the enzyme. The image was taken from the PDB (code 1BLG). (c) Evolution of the mean enzymatic activity monitored via the fluorescence intensity production at different stretching ratios during three stretching/unstretching cycles. Adapted from ref 38 with permission from The Royal Society of Chemistry.

to modulate chemical processes. There exist short peptides or artificial enzymes behaving as efficient catalysts: this property, coming from the secondary structure, was adopted by the peptide chain. Can one change the secondary structure of polypeptides by using a mechanical force such as stretching? For chains that can undergo a coil/helix transition and which are close to this transition point, a theory predicts that stretching can induce helix formation.[39−42] Until recently, this effect was observed only once for gelatin.[43] Using cross-linked PLL/HA multilayers, we showed that PLL undergoes a stretch-induced conformational change and that this change is reversible when returning to the nonstretched state. In the nonstretched state at pH 7.4, the PLL chains are in a random coil conformation while under stretching; they undergo a transition to α-helices and to the 3_{10} helix, a conformation never observed for PLL in solution.

IV. PERSPECTIVES ON SOFT-MECHANOCHEMISTRY

The dynamic nature of cells and all living organisms strongly relies on the transduction of mechanical forces into chemical signals. This is mainly done through conformational changes in macromolecular and supramacromolecular entities. The exhibition of cryptic sites in proteins under mechanical stress is one example of such changes that induce chemical processes. Soft-mechanochemistry can be defined as the area of chemistry which develops systems transducing mechanical forces into chemical processes by making use of such conformational changes. In contrast to conventional mechanochemistry, where mechanical forces affect chemical bonds, the soft-mechanochemistry approach is based on much less energy demanding processes or forces of much lower intensity. This field of mechanochemistry is still in its infancy. Until now, most of the systems designed along soft-mechanochemistry principles concern mechanoresponsive surfaces or films. These systems lead to a decrease in fluorescence, a change in enzymatic activity, or an exhibition of cryptic sites by stretching.

To further progress in this field, the first step could be to design cryptic site molecules, namely, molecules that possess an interaction site with a receptor. This site could be an adhesion site or a site that is cleavable by enzymes and accessible only to its receptor when the molecule is stretched. Until now, only surfaces possessing cryptic sites covered by shielding chains were developed. The design of cryptic site molecules would allow us to go one step further. Such molecules could be anchored on surfaces or incorporated into stretchable materials. They could form the basis of new systems that could self-assemble when submitted to hydrodynamic flow, mimicking the von Willebrand factor. A second challenge of soft-mechanochemistry would be to design catalytically active films which are inactive at rest and become active upon stretching. The few catalytic systems reported so far rely on modifying the enzyme conformation with stretching and thus decreasing its catalytic activity. It is thus much more challenging to design systems which are inactive at rest and become active under stretching (OFF/ON systems). A third challenge of soft-mechanochemistry would be to design redox-active molecules whose redox properties (the redox potential) would change under stretching. Until now, to our knowledge, no such molecules have been designed besides macromolecules containing S−S bonds.[44] It was shown that the

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reduction potential of these bonds is a function of the stretching force. Yet this leads to bond breaking, and thus under stretching the process is not reversible.

As far as potential applications of such systems are concerned, we already mentioned the potential use of cryptic site systems as cell culture substrates with easy cell detachment. This represents a real challenge in cell culture. Similarly, such surfaces may be used as regenerable protein collectors from a cocktail of proteins for purification purposes. For this application, the scaling-up has to be worked out. Cryptic site systems could also have interesting applications when coupled to optical detectors, allowing the development of simple regenerable substrates. As far as ON/OFF enzymatically active systems are concerned, they can be used in microfluidic devices to switch reactions on and off in microchambers by simple pressure application on the chamber walls. Finally, stretch-induced reactions constitute one of nature's principles to allow cells to adhere, migrate, and react to the substrate mechanical properties. These processes should thus also become key players in artificial cells that should mimic natural cell behavior. But this is when science fiction will merge into science.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b01768.

Images of some stretching devices used to perform some of the reported results (PDF)

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Notes

The authors declare no competing financial interest.

Biographies

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Fouzia Boulmedais received her Ph.D. in physical chemistry from University Louis Pasteur in Strasbourg, France in 2003. Her postdoctoral research at ETH-Z in Zürich, Switzerland and MPI in Gölm, Germany focused on the electrochemical response of multilayers of polyelectrolytes. She joined the Institut Charles Sadron in Strasbourg, France where she obtained a position as CNRS researcher in 2006. Since January 2012, she has been deputy director of Institut Charles Sadron. Her current research involves polyelectrolyte films: their application in biomaterials and tissue engineering and their buildup by an electrical stimulus.

Pierre Schaaf got an engineering degree from ESPCI (Paris) in 1982 and a Ph.D. in physical chemistry from the University of Strasbourg in 1986. He was appointed full professor in 1991 at the chemistry engineering school of Strasbourg (ECPM). Since January 2013, he has been the director of the new INSERM unit Biomaterials and Bioengineering. His research interest includes polyelectrolyte multilayers and stimuli-responsive and bioactive films.

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