# Diazo transfer for azido-functional surfaces

Preparation of azido-functionalized polymers is gaining increasing attention. We wish to report an innovative, novel strategy for azido functionalization of polymeric materials, coupling plasma technology and solution processed diazo transfer reactions. This novel approach allows the azido group to be introduced downstream of the material preparation, thus preserving its physicochemical and mechanical characteristics, which can be tailored *a priori* according to the desired application. The whole process involves the surface plasma functionalization of a material with primary amino groups, followed by a diazo transfer reaction, which converts the amino functionalities into azido groups that can be exploited for further chemoselective reactions. The diazo transfer reaction is performed in a heterogeneous phase, where the azido group donor is in solution. Chemical reactivity of the azido functionalities was verified by subsequent copper-catalyzed azide-alkyne cycloaddition.

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The preparation of new or existing materials with tailor-made functionalities utilizing tough, efficient, and orthogonal chemistry is still challenging and will demand that more and more synthetic organic chemistry is involved in materials science. "Click" chemistry[1] is a striking example of the contribution of organic chemistry to materials science[2,3]. A variety of click reactions have been reported[4], but the Huisgen 1,3-dipolar cycloaddition catalyzed by copper (I) between azides and alkynes yielding irreversibly stable 1,4-disubstituted 1,2,3-triazole[5] plays a particularly relevant role in organic synthesis and fulfils many requirements for the conjugation of polyfunctional molecules onto polymers by post-modification processes. In fact, this cycloaddition reaction is characterized by high versatility as well as high specificity and chemoselectivity in the presence of a wide variety of surrounding functional groups and solvents (ranging from both protic and aprotic organic solvents to water). These reactions feature a high thermodynamic driving force (generally above 20 kcal mol$^{-1}$), thus allowing the use of mild

reaction conditions and short reaction times. The use of Cu(I) catalysts accelerates the process, leading to quantitative coupling yields at room temperature[6]. Cu(I)-catalyzed Huisgen-type cycloadditions have been employed in a variety of applications in the field of materials science[7,8], such as the preparation of highly functionalized macromolecules[9] and the surface immobilization of (bio)macromolecules and polymers[10,11].

In order to apply Huisgen cycloaddition to materials science, suitable methods for introducing azido groups are needed. Several strategies have been proposed for the preparation of azido-functionalized polymers[12,13]; among them we can mention a general strategy consisting of the (co)polymerization of halide functionalized monomers followed by nucleophilic substitution of the halide groups by sodium azide ($NaN_3$)[14,15]. Alternatively, the azido group can be introduced via epoxide opening by $NaN_3$ on the co-polymerized glycidyl-containing monomers (i.e., glycidyl methacrylate)[16]. A second approach consists in the (co)polymerization of azido-prefunctionalized monomers[12,13]. However, both approaches suffer from a strong limitation: the necessity of using only particular functionalized monomers (halide-, epoxide-, azido-functionalized), restricts the possibility of tailoring the chemical and physico-mechanical properties of the material, which could result in the material being unsuitable for the desired application.

In the last several years, plasma treatments have been gaining popularity as a versatile tool for the surface modification of materials[17-21]. Plasma treatments can be used to introduce different functionalities onto inert surfaces (for example, polyolefins); examples of plasma modifications include the introduction of COOH and OH functionalities through air[22-25], $O_2$[26] or inert gas (Ar, He) plasmas[24,27] (in the latter case it is the post-exposure to air of the treated materials that leads to the introduction of oxygen-containing functionalities), and the introduction of amines (through, for example, $NH_3$ plasmas). The use of plasma modification techniques has some major advantages. First of all, they modify the surface of the materials without affecting their morphology and their physical and chemical bulk properties. Moreover, other techniques will often influence the mechanical properties of the outer layer of an implant. For example, wet chemical treatment of a surface will cause a partial degradation and scissions of the polymer chains at the surface, leading to a decrease of the mechanical strength and to a faster degradation. Another advantage is that plasma treatments enable uniform modification of the materials surfaces, regardless of the geometry, and can be used on complex objects. Finally, the use of hazardous solvents can be avoided, as plasma is a solvent-free technique.

Chemically, two main strategies can be used for the introduction of an azido group on organic compounds. Traditionally, the introduction of azides has been achieved by nucleophilic attack of inorganic azides (usually $NaN_3$) on appropriate leaving groups. Recently, diazo transfer from triflyl azide ($TfN_3$) to primary amines has found widespread use[28]. This approach was initially reported in the 1970s, and found large application in the 1990s. Since then, the diazo transfer procedure has

been widely exploited, especially for the modification of biomolecules such as proteins, as recently demonstrated[29].

Both procedures (nucleophilic substitution and diazo transfer) can be readily applied to the majority of chemical structures. However, while the first approach has been extensively used in materials science, to date to the best of our knowledge no examples of diazo transfer processes have been reported for the preparation of azido-functionalized material surfaces.

Hereby we propose a novel and alternative strategy for the incorporation of azido functionalities onto polymeric materials coupling plasma technology and solution processed diazo transfer reactions. Thus the first step is solvent-free, whereas the second step is performed in a heterogeneous phase in the presence of organic solvents. The approach allows for the introduction of the azido group downstream of the material preparation, preserving in this way its physico-chemical and mechanical characteristics which can be *a priori* tailored according to the desired application. Obviously, this feature represents a great advantage in comparison with the above mentioned strategies for the insertion of $N_3$ functionalities. The method is widely applicable to polymers of different natures, since plasma allows the introduction of functional groups onto different materials, even on those not presenting reactive functional groups. The long term goal is to decorate via covalent immobilization any material with (bio) active molecules; an application that has seen rapid growth in the past decade in such industries as biomedicine, textiles, microelectronics, bioprocessing, and food packaging, in addition to biosensing or bioseparation applications[30].

## A new strategy for the azido functionalization of materials

As proof of concept we applied this novel methodology to polypropylene (PP). The whole process, illustrated in Fig. 1, involves the surface plasma functionalization of PP membranes with primary amino groups (-$NH_2$ groups), followed by diazo transfer reaction for the conversion of the amino functionalities into azido groups. The amino functionalization of polyolefinic materials by means of plasma treatment has been successfully investigated in the past[31,32]. For this purpose, plasma treatments with $NH_3$ or $N_2/H_2$ mixtures and plasma polymerization of amine-containing monomers (such as allyl amine) are generally used. The literature demonstrates[31,32] that such plasmas allow the creation of surfaces with an $NH_2$ surface density ranging from 0.5 and 1.5 groups/$nm^2$, as determined by fluorescence and colorimetric assays.

PP membranes (0.2 µm pore size) were treated with an $N_2/H_2$ plasma and characterized to assess the introduction of primary amino groups, which can be easily detected with fluorescamine, a non-fluorescent compound that selectively reacts with primary amino groups yielding a fluorescent product ($\lambda_{ecc}$= 400 nm, $\lambda_{em}$= 475 nm). Hence, we dipped untreated and plasma-treated
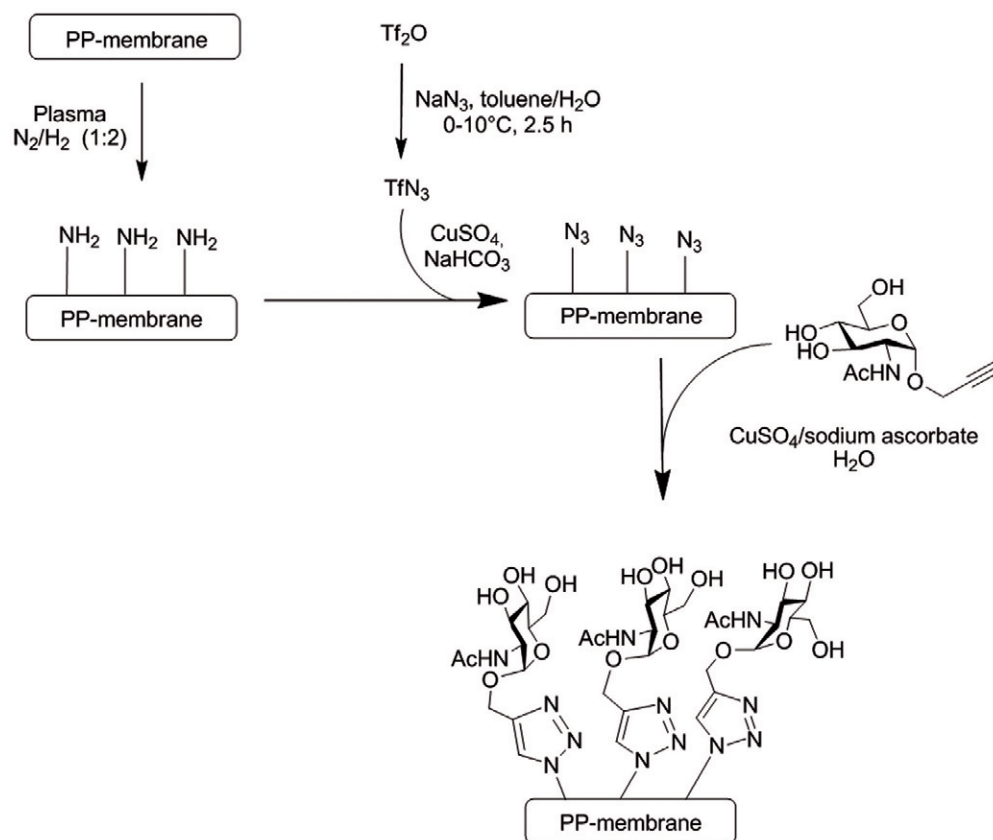
*Fig. 1 Steps towards azido-functional membranes.*

membranes in a $9 \times 10^{-4}$ M solution of fluorescamine in acetone (reaction time 10 minutes)[33]. After rinsing and drying the samples, the insertion of amino functionalities was assessed by studying the fluorescence. Figs. 2a and 2b show, respectively, an untreated and a plasma-treated PP membrane after immersion in the fluorescamine solution. Photographs were acquired under UV irradiation, using a laboratory lamp emitting light at a wavelength of 366 nm. The strong fluorescence of the modified membrane (Fig. 2b) confirms the amino functionalization due to the plasma treatment.

The conversion of the amino groups into azido functionalities was performed by a diazo transfer reaction mediated by freshly prepared triflyl azide[28]; the amino functionalized PP membrane was suspended in Milli-Q (see Methods and materials) water containing NaHCO$_3$, CuSO$_4 \cdot$5H$_2$O, triflic azide in toluene, and methanol. The total volume of the solvents was such that the membrane was fully covered, and the ratio was given by H$_2$O/toluene/MeOH, 1/1.7/6.7 such that the solvent mixture was monophasic. Attenuated Total Reflectance Fourier Transform Infrared (ATR/FTIR) spectroscopy (Fig. 3) confirms the formation of azido groups on the surface of the PP membranes. The adsorption band at 2104 nm is unequivocally assigned to the stretching vibration of the azido groups. Furthermore, a decrease of the band related to the amino functionalities was observed. The conversion

reaction is almost complete, as confirmed by the small residual fluorescence detected on azido-functionalized membrane dipped in a fluorescamine solution (Fig. 2c). In order to prove the effective diazo transfer reaction, a blank test was performed by dipping a crude PP membrane, i.e., without amino groups, in the triflyl azide solution; ATR/FTIR analysis did not show azide signals that could be ascribed to diffusion of the reactants inside the membrane.

To further confirm the presence of the azido functionality, X-Ray Photoelectron Spectroscopy (XPS) analysis was also performed (Fig. 4). N$_2$-plasma treated surfaces present several, diverse nitrogen-containing functionalities, including primary amines, amides, urethanes, etc.[32]. The best fit of the N1s peak allows us to estimate that the amine group (which is known to have a characteristic single peak at 399.3 eV)[34] accounts for 27 % (Fig. 4a, blue area) of the total nitrogen-containing functionalities (black peak). The typical peaks indicating the presence of the azido functionalities (i.e., binding energies of around 400 and 404 eV)[35], are very close to the peaks of the other nitrogen-containing functional groups, but despite the presence of several nitrogen-containing species, XPS allowed us to detect the presence of the azido functionalities (Fig. 4b, blue area). The appearance of the peak at 403.4 eV (ascribed to the electron deficient central nitrogen of the azido group)[35] is indicative of the presence of an azido group; the peak
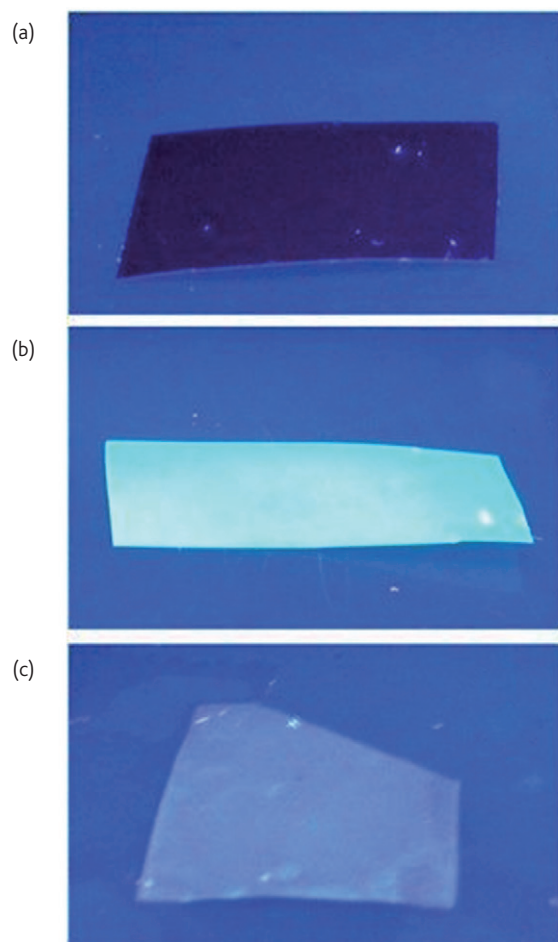
Fig. 2 Photographs, taken under UV irradiation (366 nm), of different PP membranes immersed in a fluorescamine solution. (a) Untreated membrane; (b) $N_2/H_2$ plasma treated membrane; (c) plasma treated membrane after reaction with triflyl azide.
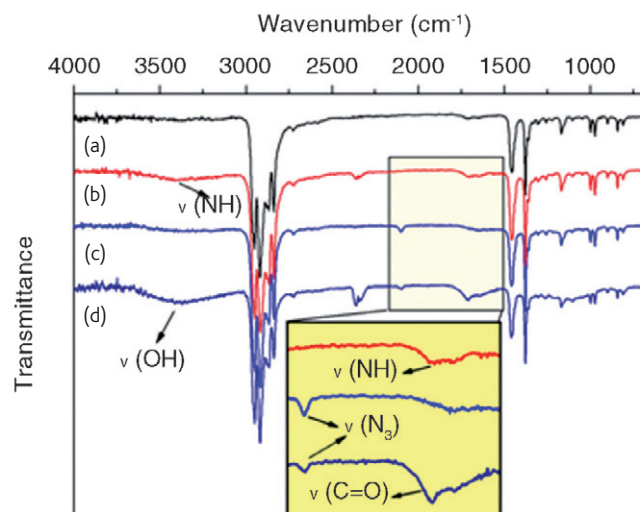


Fig. 3 ATR spectra of differently modified PP membranes. (a) Untreated membrane; (b) $N_2/H_2$ plasma treated membrane; (c) azido-functionalized membrane; (d) carbohydrate functionalized membrane by click coupling.
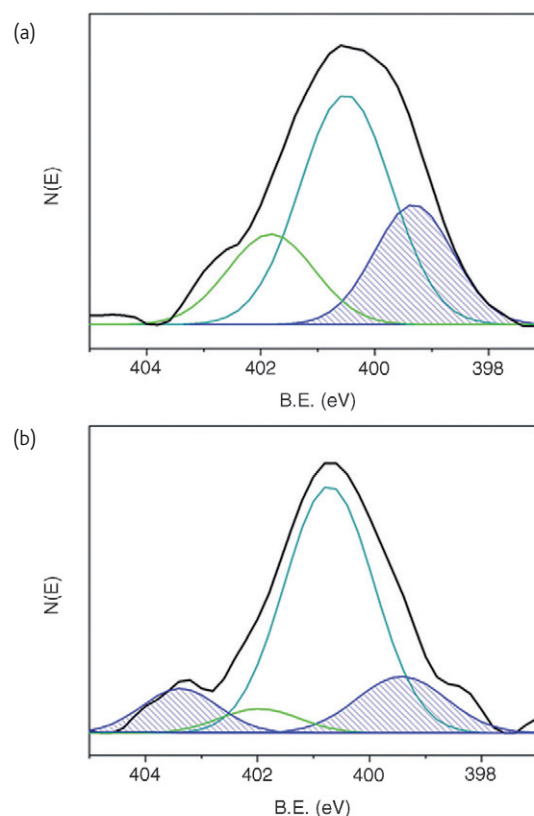


Fig. 4 XPS analysis (a) on amino-PP; (b) on azido-PP membrane.

due to the electron-rich nitrogens of the azido group is not detectable since it is hidden by peaks of other nitrogen-containing species.

The obtained azido-functionalyzed material was submitted to click coupling with an alkyne. To provide biological relevance, propargyl 2-acetamido-2-deoxy-α-D-glucopyranoside (Fig. 1) was used as a model substrate. Carbohydrates are an excellent platform for material functionalization for several reasons. First, carbohydrates can be used to tailor the material (bio)properties, for example improving cell adhesion[36], or bioactivity[37]; in addition, since they possess polar groups (i.e., hydroxyl and amino/acetamido groups) they can be used to modify the hydrophilicity of the material surface to which they are grafted.

However, since carbohydrates are polyfunctional molecules, novel and effective approaches for carbohydrate immobilization onto solid surfaces by chemoselective reactions are highly desirable. The chemoselective approach is currently finding wide application in glycomic studies[38], i.e., the study of the carbohydrate-protein interactions involved in several

biological mechanisms, including viral infection, cancer metastasis, peptide conformation, enzyme activity, cell-cell recognition, cell adhesion, and cell development. The presentation of carbohydrates in an immobilized format facilitates many such studies, allowing carbohydrate glycochips[39] to be prepared for the screening of carbohydrate arrays[40]. In this respect, alkynyl
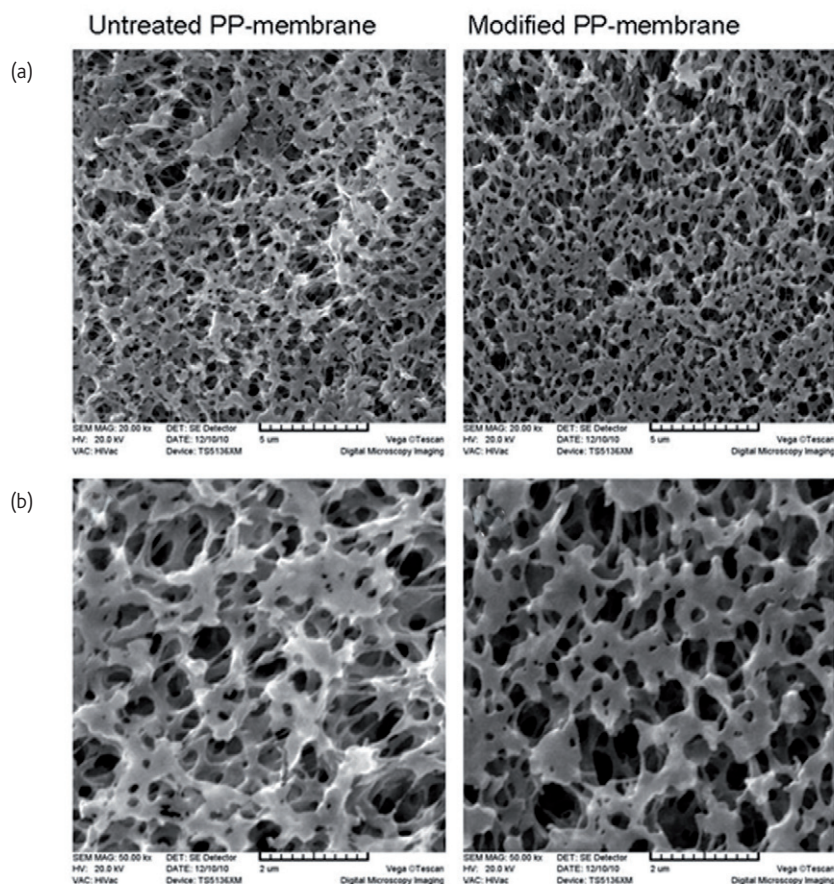
Fig. 5 SEM images of untreated and modified PP-membranes with different enlargements.

glycosides, which are suited for the chemoselective Huisgen cycloaddition, are particularly interesting since they can be easily obtained from the free parent saccharide in excellent yields and purity[41].

Propargyl 2-acetamido-2-deoxy-α-D-glucopyranoside is an interesting model substrate, since it is a relevant monosaccharide involved in many biological phenomena, and, in addition, it possesses the acetamido functionality at carbon 2, that can be exploited as a probe for ATR/FTIR surface characterization of the functionalized PP. The PP-N$_3$ surfaces were submitted to copper(I)-mediated Huisgen cycloaddition in water. ATR/FTIR spectra (Figs. 3c and 3d) confirmed the success of the coupling reaction. In fact, a decrease of the adsorption band at 2104 nm (stretching vibration of the azido groups) can be observed, together with the appearance of new bands at 3020 – 3750 cm$^{-1}$ and 1708 cm$^{-1}$ that can be ascribed to the stretching vibrations of OH and C=O groups (of the acetamido functionality) of the coupled monosaccharide, respectively.

In order to further confirm the cycloaddition reaction, contact angle measurements were performed prior to and after the coupling reaction. The azido-functionalized surface is hydrophobic (contact angle = 122° ± 4°). Instead, the carbohydrate-functionalized surface, as expected, is hydrophilic (contact angle = 74° ± 7°). This result

represents further evidence of the successful cycloaddition on the modified PP membranes.

Finally, the morphology of the material before and after surface modifications was investigated by scanning electron microscopy (SEM), that does not provide evidence for any major changes of the membrane structure and porosity (Fig. 5).

## Conclusions

In conclusion, we have shown that a two step plasma/diazo transfer surface modification can be efficiently used to functionalize the surface of polypropylene. SEM analysis does not show any substantial change in the material morphology. The azidation of polypropylene

### Instrument Citation

EL-Flow series F-201C by Bronkhorst flow meter

Nicolet Avatar 360 FT-IR spectrometer equipped with a PIKE MIRacle ATR sampling accessory

DataPhysics OCA 20 contact angle system

Perkin-Elmer PHI 5400 ESCA System apparatus

Tescan VEGA TS 5136XM scanning electron microscope

provides an ideal platform for subsequent 'click' reactions, and allows the decoration of the material with any compound presenting carbon-carbon triple bonds. The method presented is widely applicable, and allows the functionalization of materials with different natures, and a wide variety of (bio)active compounds.

## Materials and methods

*Materials.* Polypropylene membranes (0.2 µm pore size) were purchased from Whatman. All chemicals were obtained from Aldrich and used without any further purification. Deionized water was obtained from a Millipore Milli-Q dispenser. The synthesis of propargyl 2-acetamido-2-deoxy-α-D-glucopyranoside[42] as well as of triflyl azide[28] was performed as previously described. Neat triflyl azide is explosive, and can be only handled in solution[43].

*Plasma functionalization.* All plasma treatments were performed in a tubular, capacitively coupled Radio Frequency (RF) reactor, which has been previously described[44]. An RF power supplier was connected through a matching network to a copper ring placed around the cylindrical chamber. Two other copper rings, placed apart from the RF antenna, were grounded. Plasma treatments were performed by feeding the discharge with an $N_2/H_2$ mixture (1:2 partial pressure ratio, total pressure 40 Pa, power input 20 W). Prior to every plasma treatment, the reactor was evacuated up to $10^{-1}$ Pa by means of a rotary pump. After this step, nitrogen and hydrogen were introduced in the chamber. The nitrogen flow was measured directly through a flow meter (EL-Flow series F-201C by Bronkhorst) while hydrogen was introduced through a needle valve. After the plasma treatment (exposure time 4 minutes), PP samples were recovered for further modifications.

*Typical procedure for the diazo transfer reaction.* The reactions were carried out in a monophasic solvent mixture of $H_2O/TfN_3$ in toluene/MeOH, 1/1.7/6.7. The total volume used was 1.4 mL (0.150 mL/0.250 mL/1 mL), determined in order to fully cover the polypropylene membrane, fitted in a 2 mL screw capped vial. Thus the amino functionalized PP membrane (1.1 cm x 0.26 cm) was suspended in 0.150 mL of Milli-Q water containing $NaHCO_3$ (39 mg, 0.465 mmol) and $CuSO_4 \cdot 5H_2O$ (1.1 mg, 0.005 mmol). Triflic azide stock solution (0.250 mL) was added, followed by the addition of methanol (1 mL). The blue mixture was mechanically stirred at room temperature for 24 hours. Then the solvents were removed and the azido membrane was sequentially washed with toluene, water and methanol and finally dried at 50 °C for 10 minutes.

*Click reaction.* 0.01 M stock solutions of propargyl 2-acetamido-2-deoxy-α-D-glucopyranoside, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate were prepared in Milli-Q water. The cupric sulfate solution (5 mol % in respect to the saccharide, 0.040 mL, 0.0004 mmol) and the ascorbate solution (15 mol % in respect to the saccharide, 0.120 mL, 0.0012 mmol) were pre-mixed and stirred until a yellow solution was obtained. The yellow solution was then added to the azido-PP membrane suspended in the saccharide solution (0.800 mL, 0.008 mmol) in a screw capped vial. The reaction was stirred at room temperature for 24 hours, then the solvents were removed and the membrane was thoroughly washed with water and methanol and finally dried at 50 °C for 10 min. mt

## Acknowledgements

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