



## Surface coatings for solid-state nanopores

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Since their introduction in 2001, solid-state nanopores have been increasingly exploited for the detection and characterization of biomolecules ranging from single DNA strands to protein complexes. A major factor that enables the application of nanopores to the analysis and characterization of a broad range of macromolecules is the preparation of coatings on the pore wall to either prevent non-specific adhesion of molecules or to facilitate specific interactions of molecules of interest within the pore. Surface coatings can therefore be useful to minimize clogging of nanopores or to increase the residence time of target analytes in the pore. This review article describes various coatings and their utility for changing pore diameters, increasing the stability of nanopores, reducing non-specific interactions, manipulating surface charges, enabling interactions with specific target molecules, and reducing the noise of current recordings through nanopores. We compare the coating methods with respect to the ease of preparing the coating, the stability of the coating and the requirement for specialized equipment to prepare the coating.

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### Introduction

In the past two decades, nanopore-based analysis of single biomolecules or nanoparticles has undergone rapid development for the detection and characterization of DNA, proteins, viruses and synthetic nanoparticles.<sup>1–13</sup> Recent advancements include the development of the portable MinION device for DNA sequencing with protein nanopores,<sup>14,15</sup> the combination of nanopore recordings with additional modalities for sensing, characterizing, or manipulating molecules such as detecting

fluorescent molecules based on plasmonic effects,<sup>16–22</sup> recording changes in the local voltage of a graphene nanoribbon transistor,<sup>23</sup> or pulling on or holding molecules in a nanopore with optical tweezers.<sup>24,25</sup>

In most cases, the basic experimental setup to detect and characterize single molecules in nanopores comprises two compartments of electrolyte solution, a thin insulating membrane that separates these compartments, and a single pore with a diameter ranging from 1–50 nm that constitutes the only connection between the two compartments (Fig. 1A). When an electric potential difference is applied across the membrane, molecules move through the electrolyte-filled pore and cause a change in the resistance of the pore by displacement of ions (Fig. 1B). The resulting resistive pulses that

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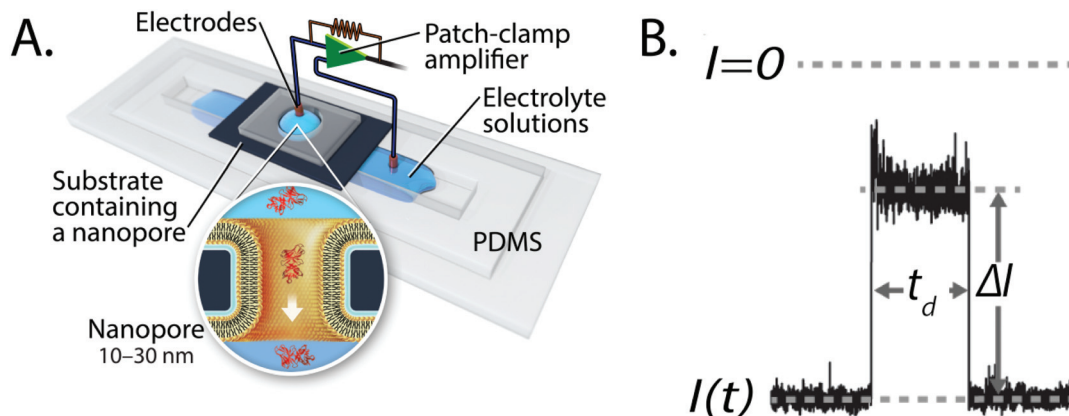
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**Fig. 1** Basic principles of resistive pulse recordings through nanopores. A. Experimental set-up for resistive pulse recordings using an electrolyte-filled nanopore; the inset shows the translocation of a single protein through a nanopore with a lipid-bilayer coating.<sup>27</sup> Figure from Yusko *et al.*<sup>27</sup> B. Example of a resistive pulse; the change in current,  $\Delta I$ , is proportional to particle volume and the dwell time,  $t_d$ , is inversely proportional to particle charge. Time-dependent modulations of the current during a resistive pulse can reflect the orientation of non-spherical particles and reveal their shape, rotational diffusion coefficient and dipole moment.<sup>27</sup>

coincide with the translocation of individual particles reveal characteristics of that molecule. For instance, the most probable dwell time of the resistive pulse is inversely proportional to the charge of the molecule, while the amplitude of the resistive pulse is related to the volume, shape, and orientation of the molecule in the electric field (Fig. 1B).<sup>26</sup>

There are two major types of nanopores: biological nanopores<sup>28–32</sup> and synthetic, solid-state nanopores.<sup>3,8,33</sup> Biological nanopores consist of transmembrane proteins that enable the translocation of molecules through their lumen.<sup>34,35</sup> The most widely used example of this type of nanopore is the  $\alpha$ -hemolysin protein that is expressed by *Staphylococcus aureus* and self-incorporates into lipid

membranes.<sup>28,36</sup> The narrowest constriction of protein pores is relatively small; their diameters typically range from 0.4 nm–3.4 nm.<sup>5,6,28,37,38</sup> These constrictions enable resistive-pulse recordings with high signal-to-noise ratio, they make protein pores attractive for the detection of analytes with at least one small dimension such as ions,<sup>39</sup> organic molecules,<sup>40</sup> peptides and unfolded proteins,<sup>41</sup> as well as for sequencing of DNA and RNA.<sup>42–46</sup> Other attractive features of these pores for biophysics and biosensing applications are the availability of crystal structures of several of these pore proteins with atomic resolution,<sup>36,47–52</sup> their evolved resistance to clogging,<sup>29</sup> their amenability to site-specific chemical modifications,<sup>53,54</sup> and the excellent reproducibility of producing these pores by established protein expression and purification methods.<sup>28</sup> Biological nanopores have, however, three main limitations: first, their small diameters prevent the ability to characterize large molecules. Second, their intrinsic fragility can lead to fluctuations of the baseline current through these pores under certain conditions such as elevated applied potential differences or elevated temperature.<sup>6,29</sup> And third, the requirement to reconstitute these proteins into a lipid bilayer or polymer membrane poses the challenge to prepare a stable lipid or polymer membrane for each experiment, and to reconstitute protein pores into these membranes efficiently<sup>55</sup> before each experiment.

The lack of large-scale tunability of the diameter of biological nanopore provided one of the motivations for the development of synthetic nanopores. Synthetic nanopores can be fabricated in virtually any size from below 1 nm in diameter<sup>56</sup> to the sub-micrometer range.<sup>57,58</sup> These sizes allow for the analysis of a large range of biomolecules including proteins, viruses, and nanoparticles.<sup>42,59,60</sup> Nanoscale pores or channels in solid state materials are fabricated by various techniques<sup>61–66</sup> including ion beam sculpting,<sup>56</sup> focused ion beam fabrication,<sup>67</sup> transmission electron microscopy,<sup>68</sup> electron beam fabrication,<sup>69–73</sup> track-etching,<sup>74–78</sup> dielectric break down,<sup>79,80</sup> laser-assisted dielectric breakdown,<sup>80</sup> laser-assisted

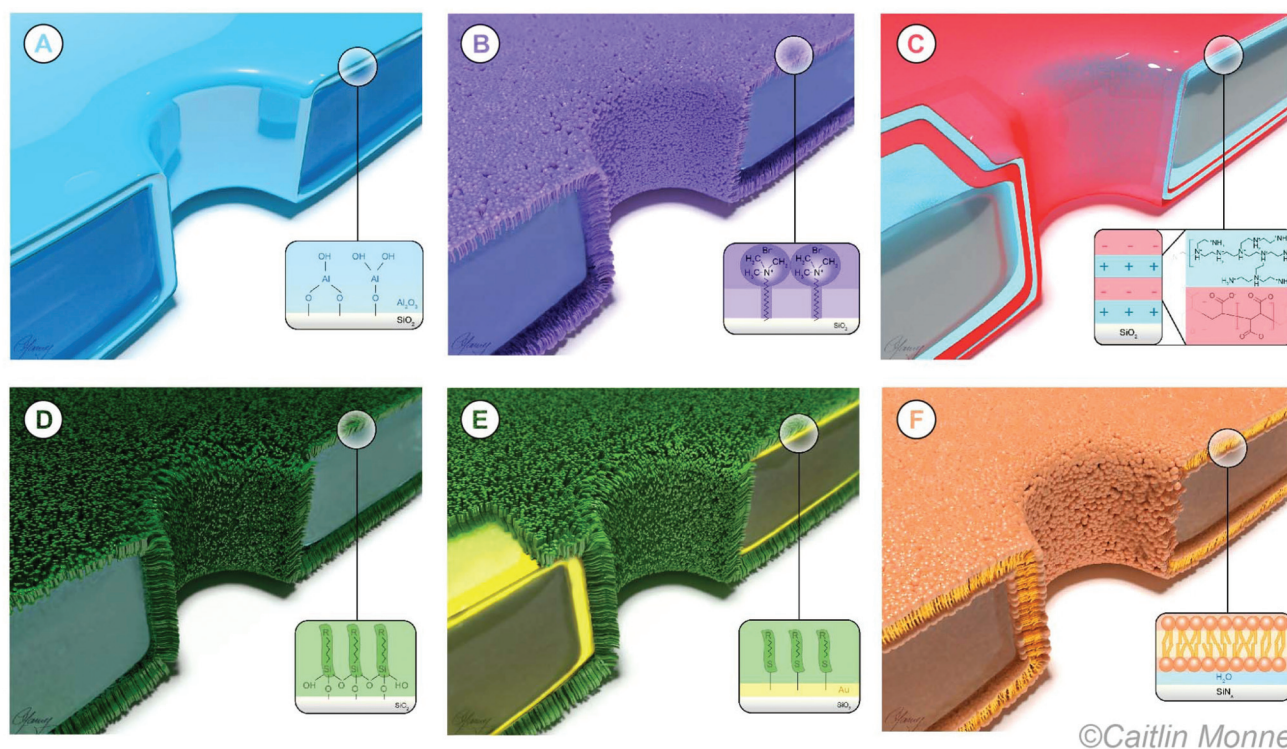


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**Fig. 2** Idealized cartoon representations of the most commonly employed nanopore coatings. A. Cross-section through a  $\text{SiO}_2$  membrane with a coating of  $\text{Al}_2\text{O}_3$  deposited on the membrane and on the walls of the nanopore. B. Coating of a nanopore prepared by physisorption of a surfactant. C. Coating prepared by layer-by-layer self-assembly of negatively and positively charge polymers. D. Coating prepared by silanization. E. Coating of a self-assembled monolayer of alkanethiols on gold. F. Coating of a nanopore with a fluid lipid bilayer.

etching,<sup>80–82</sup> and layer-by-layer removal.<sup>83</sup> These techniques make it possible to create nanopores with varying shapes<sup>84</sup> and surface chemistries<sup>85</sup> in a range of materials including silicon nitride,<sup>86,87</sup> silicon dioxide,<sup>68</sup> hafnium oxide,<sup>88</sup> aluminum oxide,<sup>89</sup> graphene,<sup>90,91</sup> glass<sup>92–94</sup> and polymer films.<sup>95,96</sup>

One major limitation of solid-state nanopores is the tendency of their walls to interact non-specifically with many analytes. These interactions can lead to clogging of the pores and to the inability to translocate additional molecules. In this context, proteins, which constitute an increasingly common analyte in nanopore-based biophysics studies, are particularly prone to interact with the walls of synthetic nanopores.<sup>97</sup> Factors contributing to these interactions include electrostatic attraction,<sup>98–100</sup> van der Waals forces,<sup>101–103</sup> and hydrophobic interactions.<sup>97,104–106</sup> Surface coatings such as those shown in Fig. 2 can help to reduce the strength of these interactions and thereby allow for unperturbed translocation.<sup>107</sup>

The negatively charged surfaces on the walls of most synthetic nanopores also lead to two phenomena that are relevant for nanopore-based analyses: electroosmotic flow (EOF) and ion current rectification (ICR). Charged surfaces in contact with a liquid electrolyte accumulate a layer of counter-ions forming an electrical double layer (EDL).<sup>108</sup> When a potential is applied that drops along the length of a nanopore, it drives electrophoretic movement of ions in the EDL creating EOF whereby the liquid moves with the ions.<sup>109–111</sup> Electroosmotic

flow provides an additional force on molecules inside the nanopore, which can either add to the electrophoretic force or point in the opposite direction, depending on the net charge of the molecule and on the polarity of the charges on the nanopore wall.<sup>112,113</sup> In the context of nanopore sensing, it is important to either minimize EOF as much as possible or to keep it constant at a well-defined level in order to analyze and interpret translocation time distributions from the motion of particles or macromolecules through the pore.<sup>27,107,114</sup>

The second phenomenon that originates from charges at the nanopore wall is ICR, which requires either an asymmetry of the pore geometry or of the distribution of surface charges along the pore's long axis.<sup>115–117</sup> The resulting asymmetric ion distribution leads to a preferential current flow towards one polarity of the applied electric potential difference compared to the other polarity, leading to non-linear curves of current as a function of applied voltage similar to an electric diode.<sup>115,118–123</sup> This phenomenon can be exploited for sensing purposes,<sup>121,124–126</sup> however for applications that benefit from a uniform electric field along the nanopore, ICR should typically be eliminated.<sup>80</sup> Surface coatings provide a way to increase, reduce or invert surface charges on the walls of nanopores.<sup>127–130</sup> Tuning the charge density on the nanopore wall or the ionic strength of the electrolyte solution adjusts the screening length of the EDL and can hence be used to manipulate the velocity of EOF in the nanopore channel.<sup>119</sup>





**Table 1** Comparison of the main benefits and characteristics of different methods for coating nanopore walls

Method	Application				Characteristics			
	Reduce non-specific interactions	Manipulate surface charges	Engineer specific interactions	Change pore diameter	Reduce noise	Ease of coating	Stability of coating	Specialized equipment
Depositions from the vapor phase <sup>82,130,140–154</sup>	•	+	•	++	+	++	++	Yes
Surfactants <sup>128,155,156</sup>	+	+	•	•	•	++	–	No
Other physisorbed surface modification <sup>157–160</sup>	+	+	+	•	•	++	–	No
Layer-by-layer self assembly <sup>161–170</sup>	+	++	+	++	•	•	+	No
Silanization <sup>129,171–191</sup>	+	+	++	+	•	+	+	No
Self-assembled monolayers of thiols on gold <sup>188,192–209</sup>	+	+	++	+	•	+	+	Yes <sup>a</sup>
Other covalent surface modifications <sup>123,127,210–254</sup>	+	+	++	+	•	+	+	No
Fluid lipid coatings <sup>27,107,114,255–261</sup>	++	++	++	+	•	–	•	No

Coatings which provide a better than average positive outcome are marked with (++), coatings which provide a positive influence on nanopore sensing are marked with (+), those with a neutral influence are marked with (•), and coatings that may incur a negative effect with regard to a certain property are marked with (–). <sup>a</sup> For a self-assembled monolayer of high quality involving a gold surface with the application of thiols, a fresh, unoxidized layer of gold is necessary, typically requiring a set up for sputtering or otherwise depositing thin films of gold.

To address problems such as limited stability of synthetic nanopores by slow “etching” in electrolyte solutions<sup>131,132</sup> or non-specific interaction of analytes with pore walls of synthetic nanopores, various coating methods have been developed (Fig. 2). These methods range from metal oxide deposition to self-assembled monolayers of thiols on gold (Table 1) and have been discussed previously for use in nanopore experiments in several excellent review articles.<sup>133–139</sup> This review provides an update as well as a comprehensive exploration of the current status of the use of surface coatings in the nanopore field. With this goal in mind, Table 1 presents an overview of the eight most common coating methods together with their suitability for various applications. Following the organization in this table, this review discusses these coatings as well as their benefits and limitations for the analysis of single macromolecules and particles. While the primary motivation and rationale for nanopore coatings is often to avoid adhesion to the pore wall, once applied, these coatings provide additional advantages, which we will discuss throughout this review.

## Types of surface coatings for synthetic nanopores

### Depositions of coatings from the gas phase

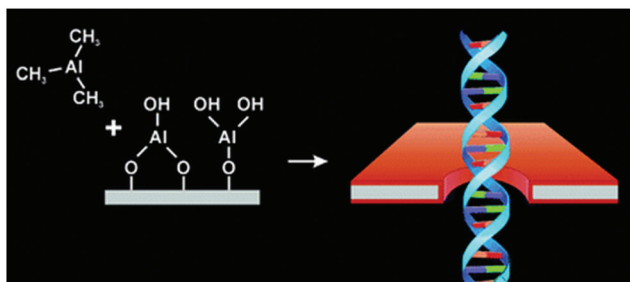
Vapor depositions by atomic layer deposition (ALD)<sup>262</sup> and chemical vapor deposition (CVD)<sup>263</sup> allow for the application of material in a well-controlled manner.<sup>142</sup> The precision, in terms of layer thickness, especially of ALD, which cycles through the deposition of individual single-molecule layers, makes gas-phase depositions an attractive technique for coating nanopore walls.<sup>264</sup> Alternatively, electron beam-induced deposition (EBID) is a technique for spatially localized deposition that involves physisorption of precursor molecules on the surface followed by deposition mediated by

electrons.<sup>265,266</sup> Potential benefits of depositions on membranes containing a nanopore, besides the obvious change in pore size and shape,<sup>140,142,143,146,147,150,151,154</sup> include reduction of recording noise,<sup>142,149</sup> modification of surface properties such as charge or hydrophobicity,<sup>130,140,142,147,148</sup> control of current rectification,<sup>152</sup> and manipulation of surface interactions with analytes of interest or with other molecules in a sample.<sup>141</sup> While depositions of coatings from the gas phase make it possible to reduce the pore diameter, the same process leads to a concomitant, and often undesired increase in nanopore length. Long pores, on the one hand, increase the dwell times of resistive pulses, thereby improving the time resolution; on the other hand, long nanopores have an increased sensing volume and thereby result in a decreased signal to noise ratio compared to shorter pores with the same diameter.

Due to the limited accessibility to recessed nanoscale features, depositions of a continuous film can be difficult to achieve inside nanopores. Elam *et al.* explored this limitation on pores with high aspect ratios (length/diameter up to 5000) by assessing the uniformity of the coatings at various locations in the pores.<sup>144</sup> These authors utilized Monte Carlo simulations to predict the necessary exposure times in a general form that could be applied to any porous substrate.<sup>144,145</sup> In order to further increase the quality of depositions in nanopores Fan *et al.* developed a dual-stage ALD process. This process led to coatings with high levels of homogeneity and conformity within the pores.<sup>153</sup>

Chen *et al.* demonstrated that controlled deposition of Al<sub>2</sub>O<sub>3</sub> by ALD is a potential strategy to reduce 1/f noise, control the diameter, and neutralize the surface charge of nanopores prepared by ion beam sculpting in silicon nitride membranes.<sup>142</sup> The authors showed that this coating increased the throughput of DNA translocations through the pore compared to a pore in uncoated SiN<sub>x</sub> membranes (Fig. 3). They attributed low throughput before deposition to a variable surface charge distribution





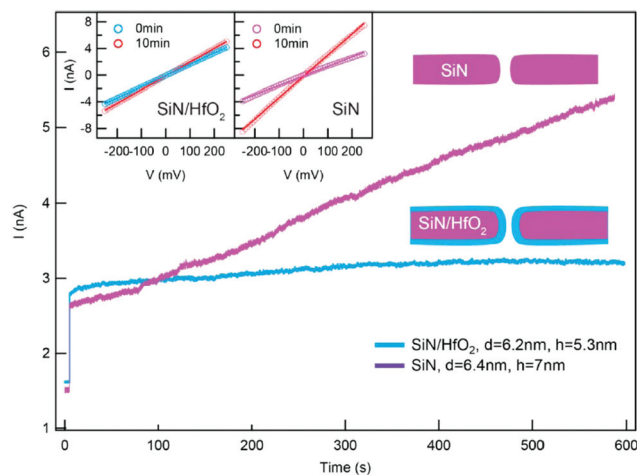
**Fig. 3** Representation of the deposition of  $\text{Al}_2\text{O}_3$  on a solid-state chip with a nanopore as an example of a coating prepared by ALD. The coating is shown in red. This  $\text{Al}_2\text{O}_3$  coating allowed Chen *et al.* to analyze DNA strands without clogging.<sup>142</sup> Reprinted with permission from ref. 142. Copyright 2004 American Chemical Society.

within the nanopore and hypothesized that the uniform surface properties after ALD coating enabled DNA analysis without clogging and long-lived or permanent blockages.<sup>142</sup>

In an effort to discriminate between single- and double-stranded DNA, Thangaraj *et al.* performed ALD of  $\text{Al}_2\text{O}_3$  on track-etched nanopores in poly(ethylene terephthalate) (PET) films to reduce the surface charge as well as to control the shape and size of these pores.<sup>130</sup> Specifically, the deposition reduced the diameter by  $\sim 25\%$  and reduced current fluctuations resulting from free polymer chains on the surface after the track-etching process. The resulting change in pore diameter and increase in aspect ratio, lowered the strength of the electric field and prolonged dwell-time in the pore, enabling the detection of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA).<sup>130</sup>

In order to reduce the diameter of nanopores, Kox *et al.* used EBID for applying a coating of a hydrocarbon compound.<sup>146</sup> The deposition shrunk the nanopore size from around 100 nm to a diameter of 20 nm and the resulting elimination of ICR suggested that the shape of the pore changed from conical to symmetric. In addition, using a  $\text{SiO}_2$  precursor in EBID led to a chemically stable and constant surface charge, facilitating the detection of biological macromolecules.<sup>146,147</sup>

One important aspect of deposition techniques on membranes containing nanopores is that they can increase the stability of the membranes, and in particular, of the pore diameter against slow etching in electrolyte solution during recordings. The long-term stability of the nanopore diameter is essential for quantitative and reproducible experiments, since a small change in nanopore diameter can induce a relatively large difference in the sensing volume, the resistance and the thermal noise of nanopores. During nanopore recordings, pores are immersed in an electrolyte solution with an applied potential difference and are often cleaned aggressively by a hot solution of concentrated sulfuric acid with hydrogen peroxide (so called Piranha solution) or by an  $\text{O}_2$  plasma between experiments. While  $\text{SiN}_x$  and  $\text{SiO}_2$  are considered chemically and physically robust materials, there is nonetheless a problem with slow etching on the nanometer scale.<sup>267</sup> Specifically,  $\text{SiO}_2$  on the nanopore walls, which originates either from membranes



**Fig. 4** Coating a nanopore in a  $\text{SiN}_x$  membrane with a layer of  $\text{HfO}_2$  effectively inhibited the growth of the nanopore diameter.<sup>82</sup> Graph showing a time-dependent recording of baseline current through an uncoated nanopore in a  $\text{SiN}_x$  membrane (purple) and through a nanopore coated with  $\text{HfO}_2$  (blue) while a voltage of 100 mV was applied and while the membrane was simultaneously illuminated by a laser (532 nm). Insets: Current versus voltage curves showing the difference in resistance of two nanopores before and after measurements that lasted for 10 min. Reprinted with permission from ref. 82. Copyright 2018 American Chemical Society.

composed entirely of  $\text{SiO}_2$  or from oxidation of the surface of  $\text{SiN}_x$  membranes, hydrolyzes slowly to silicic acid and dissolves in the electrolyte solution during recordings.<sup>131,268</sup>

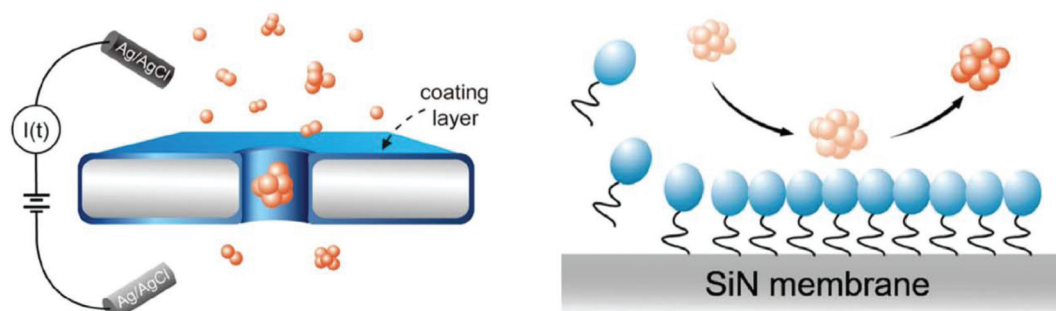
The etch rate of  $\text{SiN}_x$  or  $\text{SiO}_2$  during nanopore experiments varies as a function of temperature, pH, salt concentration, applied voltage, nanopore shape and nanopore fabrication methods<sup>132,267,269,270</sup> and can sometimes be sufficiently fast to lead to a noticeable increase in conductivity through the growing pore during the experiment. The resulting uncertainty in pore diameter, shape, volume, and electric field inside the pore leads to uncertainty in quantitative resistive pulse experiments that aim to characterize translocating particles or molecules.

One promising coating that can be applied by gas phase deposition is hafnium oxide ( $\text{HfO}_2$ ). This coating has shown high chemical stability during extended nanopore experiments.<sup>71,88</sup> For instance, by depositing a thin layer of  $\text{HfO}_2$  using ALD on the walls of a nanopore formed in a  $\text{SiN}_x$  membrane, Yamazaki *et al.* have effectively inhibited  $\text{SiN}_x$  dissolution in a photothermal etching environment (Fig. 4).<sup>82</sup> Coating nanopores with a protective self-assembled monolayer (SAM) has also been shown to prevent nanopores from slow etching in aqueous electrolyte solution and enabled measurements for several days.<sup>172,271</sup> Nonetheless, slow etching of nanopores leading to increasing pore diameters continues to be one of the major challenges in recordings with solid-state nanopores, especially when very thin insulating membranes ( $<30$  nm) are required.

### Surfactant-based nanopore coatings

Surfactants (surface-active agents) can adsorb on surfaces and alter the surface chemistry of that surface.<sup>128,272–274</sup> These





**Fig. 5** Cartoon representation of the putative effect of Tween 20 (shown in blue) on non-specific adsorption of proteins to the pore wall.<sup>155</sup> The circles represent the hydrophilic moiety while the tails represent the hydrophobic moiety of the surfactant molecules. Figure from ref. 155.

amphiphilic agents consist of both hydrophobic and hydrophilic residues, allowing, in some cases, for their unaided adhesion to surfaces.<sup>274</sup> Surfactants typically lower surface tension and may perform additional functions such as foaming, inhibiting corrosion, and killing bacteria.<sup>272,273</sup> Nanopore coatings with surfactants such as Tween 20 and cetyl trimethyl ammonium bromide (CTAB) are primarily employed to reduce interactions of biomolecules with the pore wall,<sup>155,156</sup> they can, however, also provide the ability to alter surface charge density and therefore current rectification.<sup>128</sup>

Hu *et al.* showed that the commercially-available surfactant Tween 20 prevented irreversible clogging of nanopores as a result of minimized protein adsorption to the pore wall,<sup>155</sup> Fig. 5 illustrates the proposed mode of action.<sup>155</sup> Specifically, the authors suggested that Tween 20 application to silicon nitride nanopores rendered the surface more hydrophilic (as confirmed by contact angle experiments), minimizing adhesion of the protein alpha-synuclein that is implicated in Parkinson's disease.<sup>275</sup> This modification allowed for the identification of four types of alpha-synuclein oligomers<sup>155</sup> as well as the differentiation between ssDNA and dsDNA.<sup>156</sup>

Xie *et al.* tested CTAB for generating a coating that inverted the negative surface charge of track-etched nanopores in PET foils to a surface with a positive charge.<sup>128</sup> Through the adjustment of the CTAB concentration in the solution used for recording, the authors changed the surface charge from  $-9 \text{ mC m}^{-2}$  to  $+8 \text{ mC m}^{-2}$ , and thereby tuned the properties of current rectification.<sup>128</sup>

### Other coating methods by physisorption

The physisorption of molecules to generate coatings on a membrane with a nanopore constitutes one of the most straightforward to use surface modifications. To this end, one of the most commonly employed approaches is adsorption of positively-charged poly-L-lysine (PLL) onto negatively charged surfaces. These PLL coatings were reported to block the adhesion of molecules on the pore wall,<sup>157</sup> to allow for the manipulation of surface charges,<sup>157,159</sup> as well as to engineer specific interactions. One example of a specific interaction was that of mycotoxins<sup>160</sup> and the protease thrombin<sup>158</sup> using a cross-linker that attached to the amino groups in PLL and to

cysteine residues on antibodies specific to the molecule of interest.

In the case of nanopores in graphene, Schneider *et al.* showed that the non-covalent self-assembly of a monolayer of amphiphilic molecules, which exposed hydrophilic end groups blocked hydrophobic interactions between DNA and the graphene walls of the pore.<sup>157</sup> This coating was composed of a molecule that combined a hydrophobic aminopyrene residue with a hydrophilic tetrameric ethylene glycol moiety. The pyrene moiety putatively interacted with the graphene and the ethylene glycol protruded out from the pore wall, rendering the surface hydrophilic. This modification enabled the detection of dsDNA and ssDNA with improved reproducibility,<sup>157</sup> illustrating the potential of coated nanopores in graphene sheets.

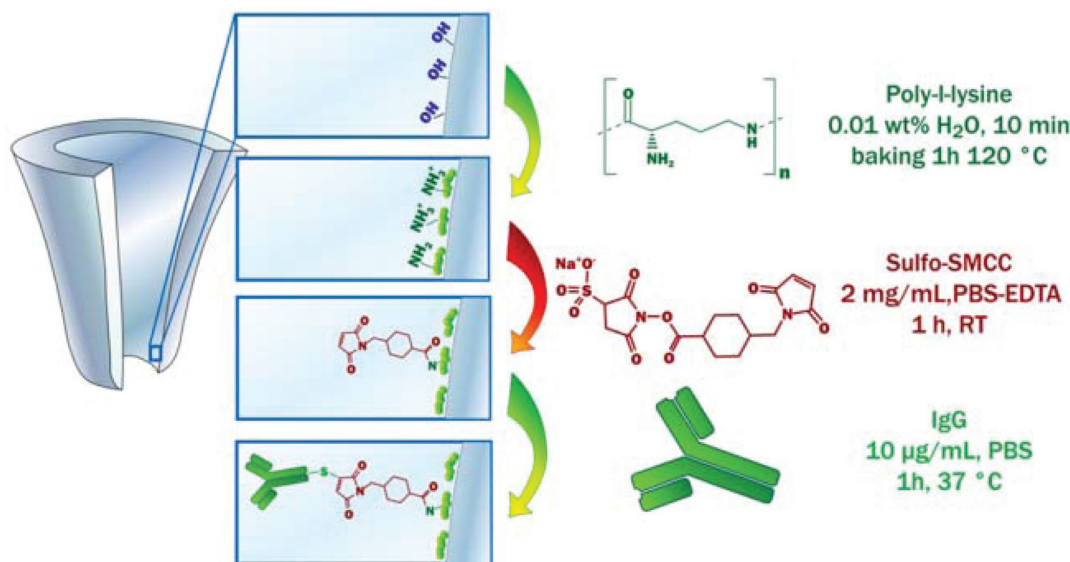
Umehara *et al.* examined the effect of PLL coatings on the mobility of ions within nanopipette electrodes.<sup>159</sup> Uncoated pipettes exhibited ICR as expected from their conical shape, while PLL-coated pipettes displayed increased rectification at the opposite polarity compared to the uncoated pipettes.<sup>119</sup> This change occurred as the positively charged PLL coating inverted the polarity of the negative surface charge of the bare glass wall of the nanopipette.

Coatings made from PLL were also used in a so-called signal transduction by ion "nanogating" (STING) sensor using a quartz nanopipette.<sup>158,160</sup> Actis *et al.* introduced this concept for the detection of the mycotoxin HT-2 by taking advantage of immunoglobulin (IgG) molecules crosslinked to the PLL coating (Fig. 6).<sup>160</sup> Immobilization of thrombin aptamers to a layer of PLL and polyacrylic acid (PAA) allowed for the detection of thrombin using the same sensing platform.<sup>158</sup>

### Coatings formed using layer-by-layer self assembly

The coating technique layer-by-layer self-assembly (LBL) employs a cycle of alternating deposition of oppositely charged polyions to create thin films.<sup>276–279</sup> These depositions typically begin with a positively charged layer to capitalize on the negative charges present on most surfaces, including glass, silicon, and metals.<sup>280,281</sup> Layer-by-layer self-assembly allows for nanoscale precision when adjusting the diameter of a nanopore since each bilayer usually contributes an increase in thickness of less than 1 nm. While the compositions of the layers and





**Fig. 6** The functionalization of a quartz nanopipette by the physisorption of PLL and subsequent immobilization of IgG molecules. This coating allowed for the detection of the mycotoxin HT-2, a small toxin that is difficult to detect.<sup>160</sup> Reprinted from ref. 160 with permission from Elsevier.

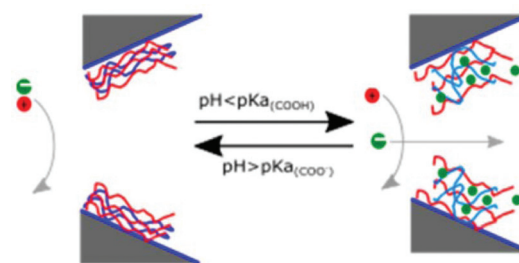
the deposition techniques vary, LBL coatings are most commonly used to manipulate nanopore size,<sup>164</sup> tailor surface chemistry,<sup>165–169</sup> or allow for the incorporation of other molecules for specific detection of certain analytes.<sup>161–163,166,167,170</sup>

In order to adjust the diameter and surface charge density of a nanopore, Lepoitevin *et al.* deposited alternating layers of PLL and poly(styrene sulfonate) onto track-etched, conically-shaped nanopores in PET.<sup>167</sup> This approach modified the pore's ICR characteristics, while the addition of PLL grafted with poly(ethyleneglycol) (*N*-hydroxysuccinimide 5-pentanoate) ether 2-(biotinylamino) ethane (NHS-mPEG-biotin) made it possible to attach or recognize biotin-binding proteins.<sup>167</sup> To design a nanopore that could be gated by the variation of pH and that responded to differences in ion concentration, Zhao *et al.* performed LBL with polyethylenimine (PEI) and chondroitin-4-sulfate (ChS) on track-etched pores (Fig. 7).<sup>168</sup>

Blundell *et al.* used layer-by-layer assembly to functionalize conical nanopores prepared in thin polyurethane membranes.<sup>166</sup> The coating made it possible to control the ionic conductance through the nanopore by changing the pH value and ionic strength of the recording electrolyte.<sup>166</sup> The authors demonstrated that layers composed of PEI and polyacrylic acid-maleic acid (PAAMA) with the incorporation of an aptamer enabled the detection 5 pM concentrations of the cancer biomarker vascular endothelial growth factor (VEGF).<sup>166</sup>

### Nanopore coatings by silanization

Silanization involves the reaction of organosilanes with surface hydroxyl groups, in a process that can be associated with molecular self-assembly.<sup>282,283</sup> Silanes comprise both organic and inorganic moieties and can form covalent bonds with varying levels of stability<sup>282</sup> on surfaces of a variety of substrates



**Fig. 7** The mechanism of a nanopore gate formed by layer-by-layer self-assembly of PEI and ChS and its response to changes in pH value of the aqueous recording electrolyte as proposed by Zhao *et al.*<sup>168</sup> At low ionic strength, the ionic current through the pore responded to changes in pH value: at pH values smaller than 4, the gate opened for flux of anions, while at pH values above 4, anion flux was significantly reduced.<sup>168</sup> Reprinted with permission from ref. 168. Copyright 2017 American Chemical Society.

including quartz,<sup>284</sup> aluminum oxide,<sup>285</sup> and iron oxide.<sup>286</sup> In the absence of polymerization, silanization forms thin coatings with low surface density, which may be used to increase hydrophobicity<sup>185,190</sup> or to reduce non-specific surface adhesion.<sup>283</sup> In the context of nanopores, silanization allows for the functionalization of pore walls by enabling the attachment of DNA,<sup>173,175,178,183</sup> dendrimers,<sup>174</sup> nucleoporins,<sup>176</sup> aldehydes,<sup>172,177</sup> spiropyran moieties,<sup>181</sup> cysteines,<sup>187</sup> carboxylic acid,<sup>172</sup> EDTA,<sup>188</sup> peptides,<sup>189,191</sup> and polymer brushes<sup>182</sup> to chemical groups that are attached to the silane molecule. Apart from the possibility of such attachments, silanization can generate a coating with antifouling properties<sup>184</sup> and can be used to manipulate ICR<sup>185</sup> and other charge-based properties,<sup>179</sup> including the modulation of surface charge by changing the pH value of the recording electrolyte<sup>129,180,186</sup>





and the regulation of transport through the conformational change of ligands in response to light or heat.<sup>171</sup>

Tan *et al.* performed silanization with 3-aminopropyltriethoxysilane (APTES) on silicon nitride nanopores (which typically have a thin layer of SiO<sub>2</sub> on their surface<sup>287</sup>) to render the net charge of the pore surface positive due to protonated terminal amine groups.<sup>179</sup> The authors chose a functionalization with amine groups to reduce EOF-related drag as well as to attract negatively charged nanoparticles to the pore entrance for detection of translocation events at increased frequency.<sup>179</sup>

Wanunu and Meller created nanopores that responded to changes in pH or to the presence of certain proteins by attaching carboxylic acid or aldehyde groups to silane coatings on the pore. To this end, they coated the pores with a variety of organosilane reagents containing epoxy, methoxyethylene glycol and amine moieties before attaching molecules that either displayed carboxylic acid groups or displayed aldehyde groups upon conjugation.<sup>172</sup> Fig. 8 shows the pH sensitivity exhibited by such a system. The coatings were formed in two ways: through (i) immersion of nanopore chips in the silane solution and (ii) voltage-driven mass transport to promote uniform coating of small pores (5 nm in diameter) without clogging.<sup>172</sup> To understand the pH dependence of selective transport of certain ions through nanopores, Wang *et al.* applied two different alkylsilanes to conical glass nanopores with a platinum disk electrode embedded at the bottom of the pore.<sup>180</sup> Specifically, a monolayer terminated in -CN groups modified the exterior surface while an amine-terminated monolayer modified the interior surface of the pore. Protonation and deprotonation of the -NH<sub>2</sub> groups affected the flux of charged species.<sup>180</sup>

To allow selective detection and sequencing of short strands of DNA through specific interactions with a binding partner in a nanopore, Iqbal *et al.* attached a hairpin loop of DNA *via* a silane layer (APTES) and a homo-bifunctional cross-linker (1,4-phenylene diisothiocyanate). The silanization of

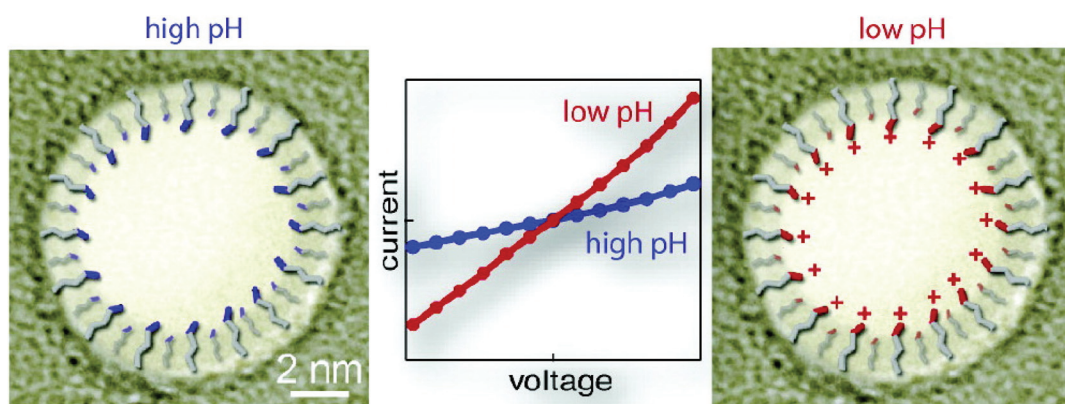
these pores also decreased their effective diameter to increase the amplitude of resistive pulses generated by DNA translocation.<sup>175</sup> Also in the pursuit of DNA sequencing, Anderson *et al.* silanized solid-state nanopores to form a 'polymeric cushion' between the DNA and the pore walls.<sup>129</sup> This cushion, composed of APTMS, prevented DNA from sticking to the nanopore walls and slowed its translocation time through the modification of the surface charge. By varying the solution's pH, the authors were able to vary the translocation times of unfolded DNA.<sup>129</sup>

Nilsson *et al.* functionalized nanopores in a silicon nitride membrane that had been prepared by focused-ion-beam drilling through a three step process.<sup>178</sup> They first grew a silicon oxide ring locally through ion-beam-assisted deposition. The oxide surface then reacted with mercaptopropyltrimethoxysilane to anchor thiol-terminated linkers. Finally, acrylamide-terminated ssDNA strands reacted with the thiol groups on the linkers, enabling detection of specific biological materials (anything from viruses to cells) through reactions with these DNA probes.<sup>178</sup> In another example, Ding *et al.* immobilized aptamers on the silanized wall of nanopores in order to render glass nanopores specific for detection of proteins.<sup>173</sup> Interaction of immunoglobulin E (IgE) and ricin molecules with aptamers in the narrow sensing zone of the pore enabled their detection.<sup>173</sup>

Tang *et al.* coated solid-state nanopores in silicon nitride membranes with polyethylene glycol (PEG<sub>200</sub>) to improve the detection of ssDNA and dsDNA.<sup>190</sup> This PEG layer lowered hydrophilicity,<sup>190</sup> 1/f noise, and the pH-dependent surface charge.

#### Coatings from self-assembled monolayers of thiols on gold

Self-assembled monolayers<sup>288</sup> are a well-studied and commonly employed approach to modify or functionalize surfaces for a variety of applications ranging from prevention of corrosion, formation of protein-repellant surfaces,<sup>289</sup> to employ-



**Fig. 8** Effect of pH-dependent surface charge of nanopores coated with a silane with protonatable end groups proposed by Wanunu and Meller.<sup>172</sup> Uncoated pores did not show the same pH-dependence as those coated with 3-(aminopropyl)trimethoxysilane (APTMS). At relatively low ionic strength (0.14 M KCl), the conductance through coated pores varied with the pH value in the recording electrolyte, while uncoated pores displayed no sensitivity to pH at either low ionic strength or at 1.0 M KCl.<sup>172</sup> Reprinted with permission from ref. 172. Copyright 2007 American Chemical Society.



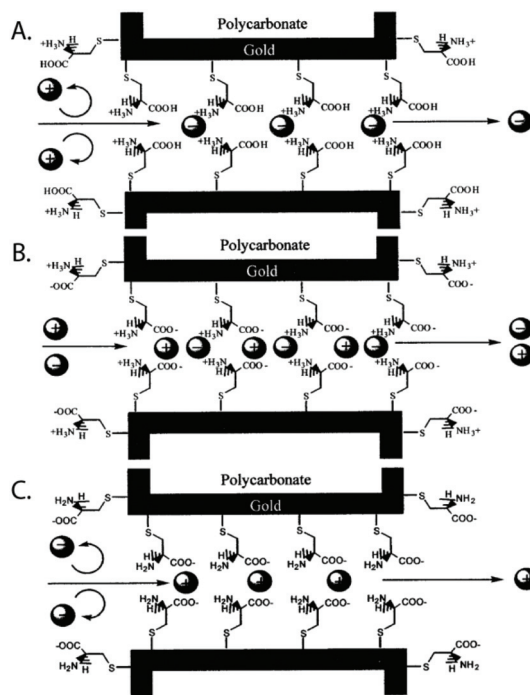


ment as active or passive elements in transistors or switches.<sup>290,291</sup> The SAMs discussed here are composed of molecules with terminal thiol groups that allow for covalent conjugation to a freshly prepared gold layer on the nanopore surface. Apart from gold deposition, SAM preparation does not require specialized equipment, the monolayers can form over large surfaces and they can provide surface groups that repel molecules, interact with, or covalently link to molecules of interest.<sup>288,292</sup> In the context of nanopore sensing, SAMs are predominantly used for sensing specific analytes,<sup>193,201,203,204</sup> minimizing non-specific interactions,<sup>188,197,200,202,208</sup> manipulating surface charge,<sup>192,194,196–198</sup> and adding functionality such as gating of the pore,<sup>192,205,206</sup> preferential transport,<sup>194,196–199</sup> or enhancing the signal of plasmonic nanopores.<sup>207,209</sup>

Charles R. Martin's group was among the first to take advantage of SAMs in nanopores by chemisorbing thiols to gold surfaces deposited onto track etched nanotubes.<sup>195</sup> The same group has since explored other modifications involving SAMs.<sup>198</sup> For example, they varied the hydrophobicity of gold nanotubules by choice of the R group in the alkane thiol molecules that they chemisorbed to the tubule walls in order to explore its influence on transport of molecules with varying hydrophobicity.<sup>196</sup> This research showed that membranes made from functionalized gold nanotubules separated hydrophobic molecules from hydrophilic species. In another study, Lee and Martin chemisorbed cysteine to gold nanotubule membranes to introduce pH-switchable selectivity for ion-transport.<sup>197</sup> At a low pH (when the cysteine's carboxyl and amino groups were protonated), the membranes allowed the passage of anions and rejected cations (Fig. 9). The opposite was true at a high pH. At the isoelectric point of cysteine (pH = 6.0), no transport selectivity was observed.

In another example of engineering specific interactions on the pore surface, He *et al.* formed a gold film on glass nanopores with ~30 nm diameters and decorated the film by self-assembly of 2-thiouracil (2-TU).<sup>193</sup> Hydrogen bonding between the amide moieties of uric acid and the 2-TU surface molecules allowed for the specific detection of uric acid, a biomarker used in the diagnosis of diseases like gout, arthritis and renal disease.<sup>293</sup> As the authors increased the concentration of uric acid in the recording buffer, the ionic current increased to a stable value, indicating the binding of uric acid to the SAM coating.<sup>193</sup>

A SAM of nitrilotriacetic acid (NTA) groups on the gold-coated walls of a nanopore enabled specific detection of His-tagged proteins.<sup>204</sup> This SAM also shrunk the diameter by ~6 nm and prevented nonspecific interactions between proteins and pore walls. Wei *et al.* demonstrated the specificity for binding of His-tagged proteins with control experiments using imidazole as a competitive binder (Fig. 10).<sup>204</sup> In another example of using nanopores in the context of protein biophysics, Jovanovic-Talisman *et al.* sought inspiration from the nuclear pore complex (NPC) and rendered the walls of nanopores in a polycarbonate film specific for transport of proteins of interest.<sup>203</sup> To recreate the NPC, the authors applied a gold



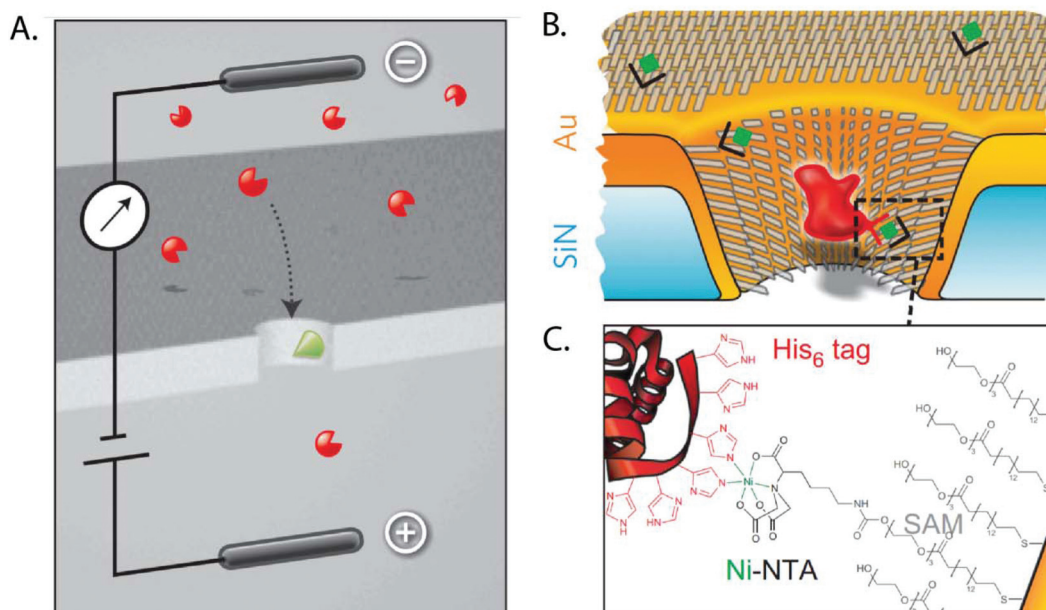
**Fig. 9** A representation of the three protonation states of cysteine chemisorbed to a gold-coated nanopore wall as proposed by Lee and Martin.<sup>197</sup> A. At a low pH, the cysteines were protonated resulting in a state that permitted transport of anions and rejected cations. B. Close to the isoelectric point of cysteine, a pH of 6, no significant selectivity for cations or anions was observed. C. At a high pH, the cysteines were deprotonated resulting in a state that permitted transport of cations and rejected anions.<sup>197</sup> Reprinted with permission from ref. 197. Copyright 2001 American Chemical Society.

layer on synthetic nanopores, attached FG-nucleoporins through a C-terminal cysteine, and attached small PEG-thiol molecules to passivate remaining areas of exposed gold. This artificial NPC effectively behaved as a filter, allowing the preferred passage of cargo in complex with transport factors specified to bind to multiple repeats of Phy-Gly (FG) motifs in the FG-nucleoporins.<sup>203</sup>

Sexton *et al.* attached PEG-thiol molecules to a gold layer prepared on the surface of track etched conical nanopores in PET membranes for the prevention of protein adsorption.<sup>202</sup> With these PEG-functionalized nanopores, the authors distinguished translocation of bovine serum albumin (BSA) in complex with anti-BSA Fab fragments from translocations of BSA alone.<sup>202</sup> This work followed a study from the same group, which exploited the advantages provided by a PEG-thiol coating to separate proteins as a function of their size by adjusting the size of the nanotubules.<sup>199</sup>

Siwy *et al.* deposited gold on conical nanopores in PET membranes to form gold nanotubes and functionalized the tubes with three different molecules for molecular-recognition.<sup>201</sup> Specifically, the authors exploited the strong interaction between biotin and streptavidin, protein G and IgG, and ricin and its antibody to increase the selectivity and sensitivity of the pores for these three analytes. The result was a simple





**Fig. 10** Cartoon depictions of the SAM-coated nanopore used for sensing specific proteins as proposed by Wei *et al.*<sup>204</sup> A. Schematic of the principle of specific analyte sensing. The proteins of interest are shown in red while the receptor that is specific to the molecule of interest is shown in green. B. The same concept shown in detail on a gold-coated nanopore with the His<sub>6</sub>-tagged proteins shown in red. C. The binding between the His-tagged protein and the SAM coating the nanopore.<sup>204</sup> Reprinted with permission from ref. 204. Copyright 2012 Springer Nature.

Boolean sensor: binding of the molecule of interest near the nanotube orifice led to a current blockage indicating the presence of the molecule.<sup>200,201</sup>

#### Other covalent surface modifications

Nanopore surfaces that expose carboxyl groups are often coated by reaction with carbodiimide moieties for coupling molecules of interest.<sup>123,127,212–234,237,238,240–247,254</sup> This technique works well on the walls of nanopores in polymer films and is robust and versatile, particularly for imparting specificity for detection of specific DNA strands or other biomolecules.<sup>217,218,223,224,227,229,232,244,254</sup> The same coupling chemistry has been employed to engineer nanopore systems that can be gated<sup>210,212,214–216,219,221,235,238,241,246</sup> or to generate nanopore diodes.<sup>123,219,232,233,239,242</sup> Other covalent modification techniques include spin-coating,<sup>252</sup> hydrosilylation,<sup>253</sup> plasma-induced graft polymerization<sup>210,211</sup> and crosslinking other functional groups such as DNA strands,<sup>235,236</sup> spiropyran,<sup>239</sup> or 4-carboxyl benzyl phosphonic acid<sup>248</sup> directly to the surface of the pore wall.

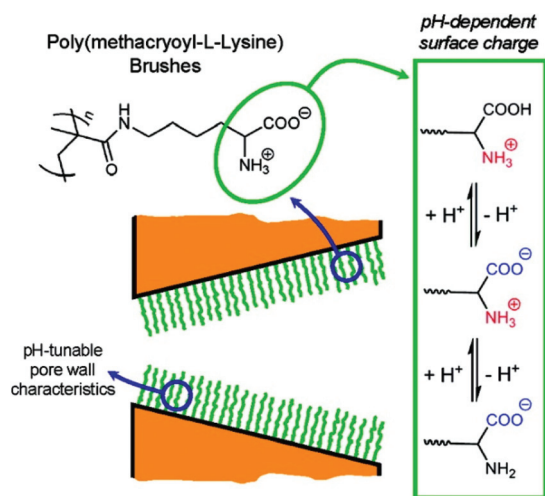
To create a pH- and voltage-sensitive mesh within a nanopore, Buchsbaum *et al.* attached ssDNA probes to the walls of conical pores in a PET film by reacting the amino groups at the 5' end of DNA oligomers with the carboxyl groups on the pore wall.<sup>212</sup> At a low pH, the DNA strands bound to each other through electrostatic interactions (AC-rich strands became protonated and GT-rich strands did not) and increased the resistance through the pore by approximately 60-fold to several tens of gigaohms. At a neutral pH, switching the polarity of the applied voltage controlled the 'gating' mecha-

nism: with the application of a negative potential, the authors proposed that the DNA strands preferentially deflected towards the smaller pore opening, causing a partial blockade, while the opposite polarity presumably caused the end of the DNA strands to move preferentially towards the larger opening, "opening" the pore.<sup>212</sup> This research group also created diodes and transistors from a nanopore in a polymer film.<sup>123,241,242</sup>

To realize a similar strategy for gating the ion flux through a nanopore, Lepoitevin *et al.* performed ALD of thin Al<sub>2</sub>O<sub>3</sub>/ZnO films on track-etched nanopores in a PET film followed by exposure of the nanopore chip to *N*-[3-(trimethoxysilyl)propyl] ethylenediamine (AEAPTMS) vapor. This treatment generated –NH<sub>2</sub> groups on the surface. Finally, they linked biotin-PEG molecules to the pore walls through AEAPTMS grafting to the surface –NH<sub>2</sub> groups.<sup>213,214</sup> Changes in pH resulted in changes in the resistance of the nanopore or, after functionalization of the biotin-PEG layer with the proteins avidin or streptavidin, this system detected biotinylated IgG, and biotinylated BSA.<sup>213</sup> Finally, the same group applied a PEG layer on the walls of nanopores in a PET film through linking to carboxylate groups on the pore surface to enable the detection of amyloids without clogging<sup>247</sup> or they attached PEG-spiropyran to the same pores to generate a light- and pH-responsive nanopore.<sup>246</sup>

Inspired by biological ion channels, Brunsen *et al.* functionalized a mesoporous thin film of silica with polymer brushes composed of poly(methacryloyl ethylene phosphate) (PMEP) to modulate ion transport by changing pH. The polymer brushes either interacted with or repelled each other depending on the pH.<sup>251</sup> Yameen *et al.* explored this concept on conical nanopores by influencing ion flow based on thermally-controlled





**Fig. 11** Cartoon depiction of nanopores with a coating of polymer brushes whose ion transport properties could be tuned by changes in pH.<sup>249</sup> Reprinted with permission from ref. 249. Copyright 2009 American Chemical Society.

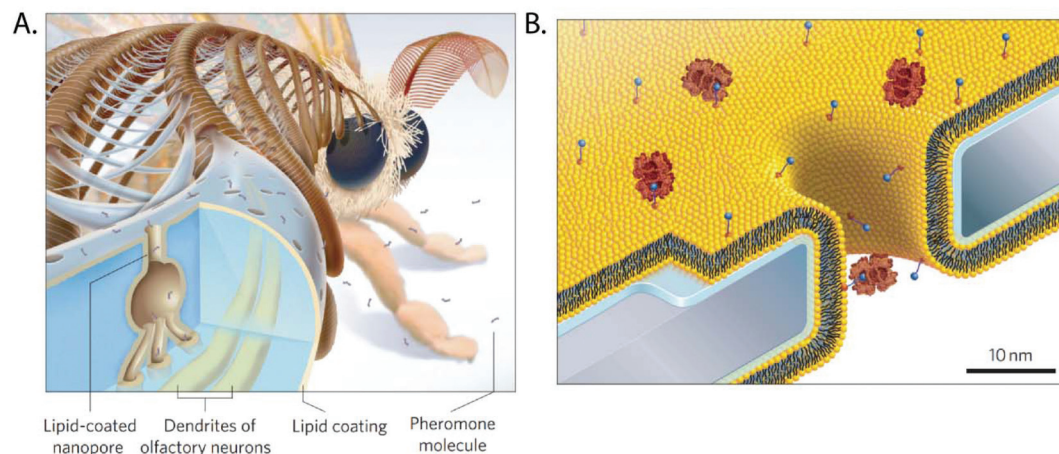
gating (Fig. 11).<sup>215,216,249,250</sup> Briefly, at room temperature the polymer brushes were in a swollen state while an increase in temperature past the critical solubility level caused the brushes to switch to the collapsed state, increasing the nanopore's effective diameter.

Ali *et al.* constructed a device for the detection of ssDNA oligonucleotides through carbodiimide-mediated coupling of specific peptide nucleic acid (PNA) probes to the surface of track-etched nanochannels in polyimide membranes.<sup>217</sup> These uncharged PNA probes also decreased the pore's ICR by about 70% compared to the ICR before modification.<sup>217</sup> Using the same technique to immobilize aptamers designed to selectively bind the enzyme lysozyme, this group locally anchored lysozyme onto the pore surface to accumulate charge and

increase ICR. Lysozyme has a high isoelectric point of 11.4,<sup>294</sup> therefore the molecules were positively charged under the experimental conditions.<sup>218</sup> Kececi *et al.* modified PET membranes by reacting surface-exposed  $\text{COO}^-$  groups with ethanolamine through (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride, EDC) coupling chemistry to reduce surface charge.<sup>127</sup> The authors used these nanopores to detect short DNA strands and to distinguish between strands of different lengths.

### Fluid lipid coatings

Coatings from fluid layers of lipids are attractive because they solve several of the most common problems in the context of nanopore recordings of proteins and other macromolecules.<sup>107,114,295</sup> Inspired by the lipid-coated nanopores present in the antennae of silk moths<sup>296,297</sup> (Fig. 12A), our group demonstrated, for instance, that lipid coatings efficiently prevent or minimize non-specific adsorption of proteins to the pore wall, eliminating clogging.<sup>27,80,107,114,258,259</sup> In addition, lipid coatings make it possible to imbue the coating with the capability to engage in specific interactions with target analytes by the incorporation of lipid-anchored ligand or receptor molecules. Binding of proteins of interest to these ligands or receptors tethers them to the bilayer (Fig. 12B) but due to the fluid nature of this lipid coating, lipid anchored target proteins can still move and translocate through lipid-coated nanopores. Depending on the strength of the interaction, these lipid anchors can concentrate molecules of interest onto the fluid coating, increasing the sensitivity of detection.<sup>107</sup> Alternatively, proteins can be cross-linked covalently to lipid anchors<sup>27</sup> or proteins such as GPI-anchored proteins, which are intrinsically lipidated, can be examined.<sup>107</sup> Lipid anchors provide the advantage to slow down the speed of translocation of the anchored target molecules by two orders of magnitude as a result of the drag of the anchor in the viscous fluid lipid coating. Finally the zwitterionic nature of



**Fig. 12** Lipid coated nanopores and their inspiration.<sup>107</sup> A. The nanopores through the exoskeleton of the sensilla in the antennae of the silk moth are lipid-coated to facilitate diffusion of pheromone molecules towards their receptors on the dendrites of olfactory neurons.<sup>299</sup> B. Cartoon of a lipid coated (yellow) nanopore with proteins (red) anchored to lipids presenting a ligand (blue) on their headgroup. The water layer with a thickness of  $\sim 1$  nm between the supported lipid bilayer and the chip surface is indicated in blue.<sup>107</sup> Figure from ref. 107.





lipids with phosphatidylcholine head groups in the coating almost completely eliminates electroosmotic flow<sup>107,114,255,256</sup> and eliminates or minimizes non-specific interactions with many proteins.<sup>27</sup>

Fig. 12 shows a schematic of a nanopore coated with a phospholipid bilayer. In one example, our group took advantage of lipid coatings to investigate the aggregation of amyloid- $\beta$  oligomers, which are associated with neurodegenerative diseases such as Alzheimer's disease.<sup>259,275</sup> Without the fluid lipid coating these experiments typically ended within seconds because amyloid- $\beta$  samples clogged uncoated nanopores in silicon nitride membranes<sup>298</sup> while lipid coatings enabled recordings for more than 40 min. More recently, we took advantage of the reduced translocation speed of lipid-anchored proteins to characterize them on the single molecule level by determining their shape, rotational diffusion coefficient, dipole moment, and charge.<sup>27</sup> For these applications, the lipid coating is essential because, on the one hand, it slows down the rotation and translocation of lipid-anchored proteins sufficiently to time-resolve their rotational motion in the pore<sup>27,295</sup> and, on the other hand, the coating provides a non-stick surface that enables translational and rotational motion of the protein without artifacts from non-specific adsorption.<sup>295</sup> Artifact-free rotation is required to quantify a protein's rotational diffusion coefficient as well as its bias towards certain orientations in the electric field inside the nanopore; it is this bias that we used to estimate the dipole moment of individual proteins.<sup>27,295</sup> We compared other nanopore coatings such as silanization and surfactant coatings with Tween 20<sup>295</sup> to lipid coatings but in our hands these alternative coatings did not eliminate wall interactions and led to artifacts.

Our group explored a variety of lipid compositions with regard to their benefits in fluid lipid coatings.<sup>114</sup> To this end, we evaluated the lipid coatings based on four major characteristics: stability of the recorded current baseline, current noise, ability of the coating to slow down the speed of translocation, and ease of preparing a stable nanopore coating. We concluded (Table 2) that the best coatings were either composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with 0 to 20 mol% cholesterol or of monolayer-forming lipids inspired from Archaea with 0 to 40 mol% POPC. These tethered lipids provide the advantage that they result in fluid lipid coatings with approximately 10-fold increased viscosity com-

pared to POPC coatings; these viscous coatings resulted in the longest translocation times of lipid-anchored proteins that we could achieve to date. One limitation is that Archaea-inspired, monolayer-forming lipids are not readily available.

Venkatesen *et al.* applied the concept of coating nanopores in SiN<sub>x</sub> membranes with lipid bilayers to coating nanopores in free-standing membranes of aluminum oxide (Al<sub>2</sub>O<sub>3</sub>).<sup>257</sup> Specifically, the authors used the liposome rupture technique with high osmotic pressure in the presence of Ca<sup>2+</sup> ions to coat Al<sub>2</sub>O<sub>3</sub> that had been deposited by ALD. The deposition of lipid bilayers composed of 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine onto membranes with single nanopores increased the impedance from less or equal to 1 M $\Omega$  to more than 1 G $\Omega$  and allowed for the integration of a biological nanopore into the lipid membrane for the formation of a hybrid nanopore.<sup>257</sup>

Hernández-Ainsa *et al.* showed that lipid coatings can also be applied onto the walls of nanopores in quartz nanocapillaries.<sup>258</sup> In this case, the lipid bilayers increased the ratio of  $\lambda$ -DNA detection from 13% to 40% presumably due to the reduction of surface charges and minimization of non-specific adsorption of DNA to the coated capillary walls.<sup>258</sup>

Galla *et al.* demonstrated that applying a zwitterionic supported lipid bilayer to nanopores prepared with a helium-ion beam in silicon nitride membranes almost completely eliminated EOF.<sup>255</sup> The authors threaded a single molecule of dsDNA through the pore with the help of optical tweezers and measured the effect of the lipid coating on the threading force. They found that the lipid coating almost completely eliminated EOF leading to an increase in threading force by 85%.<sup>255</sup> Sischka *et al.* compared lipid-coated nanopores to carbon nano membranes (CNMs) and to uncoated silicon nitride membranes.<sup>256</sup> These CNMs increased threading forces of DNA by 15% compared to uncoated membranes, showing a slight reduction in EOF, but this reduction was significantly smaller compared to the one enabled by lipid coatings.

When using nanopores in graphene for protein and nanoparticle translocation, Shan *et al.* detected gold nanoparticles after oxygen plasma treatment but they were not able to detect ferritin proteins after treatment of their graphene membranes with either oxygen plasma or mercaptohexadecanoic acid.<sup>260</sup> In order to prevent ferritin adhesion to the pore walls, they modified their graphene membranes with the nanopore by immersion in an aqueous solution of the phospholipid-PEG

**Table 2** Comparison of different lipid coatings with regard to their ability to form stable coatings with low current noise during nanopore recordings as well as their ability to slow down the speed of translocation of lipid-anchored analytes. Table from Eggenberger *et al.*<sup>114</sup>

Lipid composition of coating	Stable baseline	Low noise	Slow translocation	Straightforward to coat
100% POPC	+	+	+	+
25, 50, 80, 90, 100% Archaea lipids + 75, 50, 20, 10, 0% POPC	+	+	++	+
100% DiPhyPC	--	--	+	+
100% Di-O-PhyPC	--	--	+	+
60% DOPC + 20% DOPE + 20% LysoPC	+	-	+	+
10, 20, 30, 40% cholesterol + 90, 80, 70, 60% POPC	+	+	+	+
50% cholesterol + 50% POPC	+	+	+	-



amphiphile DPPE-PEG750; this treatment facilitated translocation and detection of ferritin but not BSA.<sup>260</sup>

To facilitate the free movement of proteins held within a nanopore by attaching them to a DNA-origami scaffold,<sup>261</sup> Schmid *et al.* coated solid-state nanopores in SiN<sub>x</sub> membranes with a lipid bilayer. In this set up the fluid lipid bilayer made it possible to observe a single molecule over extended times inside a nanopore while minimizing non-specific interactions with the pore wall.<sup>261</sup>

## Outlook

The application of a coating to the walls of nanopores makes it possible to address many of limitations that come along with approaches for the detection and characterization of single molecules in synthetic nanopores. For instance, artifacts as a result of adhesion to the pore wall, ICR and EOF can be minimized or enhanced by choice of the appropriate coating. This review outlined the spectrum of approaches to nanopore coatings as well as the resulting benefits and opportunities. While no single coating technique solves all of the problems associated with solid-state nanopores – clogging, instability, unresolved translocation events, or success rate of preparing stable coatings of high quality still present challenges for many nanopore experiments – the coatings reviewed here increased the specificity, sensitivity, versatility, and information content from nanopore-based single molecule experiments. We hope that this overview will be helpful for solving or minimizing some of the problems that hamper the usefulness of nanopore-based analytics of complex, real world samples.<sup>275</sup> We predict that the nanopore field will continue to expand the strategies for increasing the functionality of nanopores<sup>300</sup> and nanocapillaries<sup>301–303</sup> and that coatings will play an essential role in this development. We expect to witness an increase, both in the number of ways how coatings will be applied, and in the fine-tuning of their molecular composition. Another development of interest may be coatings that enable and stabilize, hybrid biological-synthetic nanopores in which at least a selection of protein pores may be tightly embedded into a coated solid-state nanopore while maintaining their full functionality.<sup>304–311</sup> Nanopores with coated walls will likely be useful for studies that manipulate or measure the forces acting on molecules during their translocating through pores, including studies that employ a combination of pressure and voltage<sup>312</sup> or laser-based trapping.<sup>313</sup> Coatings may also become increasingly important for experiments that explore nanopores in membranes made from novel materials or for nanopore studies with unconventional or non-aqueous recording electrolytes or solutions.<sup>23,314–316</sup> We are convinced that nanopore coatings will not only continue to improve the functionality of pores but will also provide a means to characterize the pores themselves, for instance with regard to their size and geometry.<sup>80,317</sup> With improved coatings, we hope that nanopore-based biophysics and analytics will continue to make a growing contribution to our understanding of biological

macromolecules and their interactions as well as to the detection of clinically-relevant biomarkers.<sup>318,319</sup>

## Conflicts of interest

Michael Mayer is an inventor on a patent application about fluid lipid coatings for nanopore experiments.

## Acknowledgements

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