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Single-sulfur atom discrimination of polysulfides with a protein nanopore for improved batteries

Fanny Bétermier¹,²,³,⁷, Benjamin Cressiot⁴,¹,⁷, Giovanni Di Muccio⁵, Nathalie Jarroux⁵,²,³, Laurent Bacri²,³, Blasco Morozzo della Rocca⁶, Mauro Chinappi⁵, Juan Pelta²,³,⁷✉ & Jean-Marie Tarascon¹,²,⁷✉

Research on batteries mostly focuses on electrodes and electrolytes while few activities regard separator membranes. However, they could be used as a toolbox for injecting chemical functionalities to capture unwanted species and enhance battery lifetime. Here, we report the use of biological membranes hosting a nanopore sensor for electrical single molecule detection and use aqueous sodium polysulfides encountered in sulfur-based batteries for proof of concept. By investigating the host-guest interaction between polysulfides of different chain-lengths and cyclodextrins, via combined chemical approaches and molecular docking simulations, and using a selective nanopore sensor inserted into a lipid membrane, we demonstrate that supramolecular polysulfide/cyclodextrin complexes only differing by one sulfur can be discriminated at the single molecule level. Our findings offer innovative perspectives to use nanopores as electrolyte sensors and chemically design membranes capable of selective speciation of parasitic molecules for battery applications and therefore pave the way towards smarter electrochemical storage systems.

¹ Collège de France, Chimie du Solide et de l’Energie, UMR 8260, 75231 Paris, Cedex 05, France. ² Réseau sur le Stockage Electrochimique de l’Energie (RS2E), FR CNRS 3459, 80039 Amiens, Cedex, France. ³ Université Paris-Saclay, Univ Evry, CNRS, LAMBE UMR 8587, 91025 Evry, France. ⁴ CY Cergy Paris Université, CNRS, LAMBE UMR 8587, 95000 Cergy, France. ⁵ Dipartimento di Ingegneria Industriale, Università di Roma Tor Vergata, Via del Politecnico 1, 00133 Roma, Italy. ⁶ Dipartimento di Biologia, Università di Roma Tor Vergata, Via della Ricerca Scientifica 1, 00133 Roma, Italy. ⁷ These authors contributed equally: Fanny Bétermier, Benjamin Cressiot. ✉ email: juan.pelta@univ-evry.fr; jean-marie.tarascon@college-de-france.fr
Batteries, as one of the most versatile energy storage technologies, play a central role in the ongoing transition from fossil fuels to renewable energy. Among them, the Li-ion batteries are the technology of choice as they offer the largest energy density while their cost is continuously decreasing. Nevertheless, improvements are needed to increase their lifetime and sustainability. Hence the ongoing activities center on alternative technologies (Na-ion, Li-air, Li–S,...) but also in the quest of means to enhance present batteries lifespan, durability and reliability. The latter calls for the development of smart batteries embedded with intelligent sensing and curing chemical functionalities.

Great advances have been made over the last few years to either restore the electrode conductivity or at the electrolyte-membrane component to regulate species migration when injured. Not surprisingly, most of the auto-repairing approaches are inspired by biological systems and benefit from the general strategies and formalisms well established for most living creatures. Following nature’s strategy relying on the use of sacrificial weak bonds for self-repairing, battery scientists have developed materials based on biomolecules or polymers, with auto-repairing properties relying on dynamic supramolecular self-assembly such as hydrogen bonding, ionic bonding or host-guest interaction.

Among supramolecular materials, cyclodextrins have been extensively studied because of their rich molecular recognition that is temperature dependent and their wide range of functionalization. Thus the feasibility to use the temperature as a stimulus to regulate the uptake or release of trapped species within their cavities, offers an interesting auto-repairing function. Such properties have already been widely exploited for thermal switches and in medicine for drug delivery as well as, with the use of heat-sensitive binders integrating sliding ring rotaxanes, self-assembled architectures of cyclodextrins to auto-repair fractured Si electrodes. Such supramolecular structures were also used as ionic conducting polymer membrane in Li-ion batteries and cyclodextrins trapping properties were exploited by placing cyclodextrins polymers in S-based electrodes to address the redox shuttle issue in Li–S batteries, e.g., migration of soluble polysulfides Li2S2(n ≤ m ≤ 8), intermediates back and forth between the two electrodes. Alternatively, other strategies to tackle the polysulfide shuttle effect have focused on the separator to prevent diffusion to the anode with either micro intrinsic porosity or using grafting chemistry to repeal via repulsive electrostatic interactions.

Up to now, existing analytical techniques to identify intermediate polysulfides and monitor Li–S batteries electrolyte composition, such as UV-vis, X-ray, mass spectrometry or cyclic voltammetry do not allow to detect and sequence at the single sulfur atom level. As the weight average chemical shift of both populations. It is fast compared to the NMR time scale, the peaks assigned to free protonation equilibrium. As the exchange rate of this equilibrium is very slow, only peaks due to the cyclodextrin cavity can be hypothesized. Therefore, we mainly focused on H3 to make the following quantitative analysis.

**Results**

Cyclodextrins reversibly store and deliver polysulfides guest. Monitoring the outcome of polysulfides species in aqueous or non-aqueous (Li, Na) metal batteries has been of paramount importance. Few approaches have been tried. They consist in targeting polysulfide migration by trapping them at the positive electrode via confinement (mesoporous carbons) or surface adsorption (oxides) means, or by tuning the electrolyte composition, or using chemically-grafted entangled separators. Because of the limited success of these approaches we have looked for an alternative with enhanced selectivity in their entrapping. In light of a recent work on the supramolecular interaction between β-cyclodextrin and sodium or lithium polysulfides, we further explore the cyclodextrin benefits towards polysulfide trapping. Our strategy consists in developing a methodology to quantify and understand the supramolecular interactions between different cyclodextrins (α, β, γ) and different polysulfide chain lengths in aqueous media, which has not been achieved yet.

To do so, several aqueous polysulfides solutions were prepared in an aqueous buffer by mixing in stoichiometric ratio Na2S with S under Argon atmosphere. The sulfur chain lengths were varied in order to get Na2S2, Na2S3, Na2S4, Na2S5 via a solution process (see “Methods” section) and characterized by UV-vis spectroscopy as previously reported (Supplementary Fig. 1). The interaction between polysulfides and the different cyclodextrins was determined by 1H NMR following the chemical shifts of the different protons of cyclodextrins in presence of polysulfides. Figure 1b shows the 1H NMR spectrum of α-cyclodextrin and Na2S4 in the deuterated buffer (red) with respect to the spectrum of β-cyclodextrin alone in the same buffer (blue). In addition, a 2D HSQC experiment was performed for signal attribution of the different protons of the cyclodextrins (Supplementary Fig. 2). The chemical shifts differences indicate the existence of a complexation equilibrium. As the exchange rate of this equilibrium is very fast compared to the NMR time scale, the peaks assigned to free and complexed cyclodextrin populations are merged and appear as the weight average chemical shift of both populations. It is worth noticing that the most shifted protons turn out to be the ones pointing inside the cavity of the host molecule such as H3 (Fig. 1bii and Supplementary Fig. 3). In this way, the host-guest interaction through the inclusion of Na2S3 inside the β-cyclodextrin cavity can be hypothesized. Therefore, we mainly focused on H3 to make the following quantitative analysis.
Chemical structures and geometric dimensions of α-cyclodextrins, β-cyclodextrins, and γ-cyclodextrins. As macrocyclic ring molecules composed of, respectively, 6, 7, or 8 glucopyranoside units, α-cyclodextrins, β-cyclodextrins, or γ-cyclodextrins can adopt their cone-truncated conformation whose inner and outer diameters vary, respectively, from 5.7 to 9.5 Å and 13.7 to 16.9 Å. The primary hydroxyl groups are located on the narrower rim of the molecule and the secondary ones are on the wider one.

Similarly, experiments with α-cyclodextrins and γ-cyclodextrins were performed and the existence of an equilibrium complexation was confirmed as well (Supplementary Figs. 2 and 3).

To go deeper in the characterization of the complex, continuous variation method, also known as Job’s plot, was performed to determine the stoichiometry of the studied supramolecular complexes. Figure 2a shows the Job’s experiments for the different cyclodextrins (α, β, γ) with Na₂S₅. Thus, the (1:1) stoichiometry for the inclusion complexation which corresponds to the Σ ratio of 0.5 was validated. We also performed molecular docking calculations in order to support our findings. Docking results show that in all cases polysulfides bind inside the cyclodextrins cavities, as shown in Supplementary Fig. 4 and Fig. 3c for β-cyclodextrin. Furthermore, regarding α-cyclodextrins and β-cyclodextrins, there is room for only one polysulfide guest per cyclodextrin host (see Supplementary Fig. 5). This is in full agreement with the NMR experiments. It can be recalled that reversible supramolecular aggregation relies on weak bonds: here both the curvature of the Job’s plots (Fig. 2a), indicative of weak binding, and the affinity scores evaluated in the docking runs (Supplementary Fig. 4) are consistent with this picture. At the same time these results, hinting strongly a one-to-one stoichiometry, provided us with a 2-states thermodynamic equilibrium model as illustrated in Fig. 1biii. Then we carried out 1H NMR titration experiments (see “Methods” section) by fitting the H₃ NMR chemical shift of the different cyclodextrins with the corresponding binding isotherm model in order to calculate the different association constants for the complex formation of Na₂S₅ with α-cyclodextrins, β-cyclodextrins, and γ-cyclodextrins (Fig. 2b). Comparable values to the previous reports were found and interestingly, β-cyclodextrin turns out to have the best affinity towards Na₂S₅ with an association constant of K_{β5} = 181 ± 4 M⁻¹, almost three times larger than with α-cyclodextrins and γ-cyclodextrins, K_{α5} = 62 ± 11 M⁻¹ and K_{γ5} = 52 ± 9 M⁻¹, respectively. Molecular docking affinity ranking over all the Na₂S₅/β-cyclodextrin inclusion complexes led to the same result highlighting the stronger affinity for the β-cyclodextrin (Supplementary Fig. 4). Hence our experimental and numerical results confirm previous literature reports. Although the Van’t Hoff plots are useful for spotting an exothermic complexation phenomenon, they fall short in sensing the difference in the strength of complexation of α-cyclodextrins or β-cyclodextrins towards Na₂S₅ in water, noted by our Molecular Dynamics simulations, as well as the geometrical complementarity issues, to name a few, which likely play a role in this complex phenomenon (Supplementary Figs. 7 and 8).

Tracking the most stable β-cyclodextrin inclusion complexes, we investigated different sizes of polysulfides. To do so, we coupled titration experiments with molecular docking calculations. As shown in Fig. 3a, a quite strong correlation is exhibited between the length of the sulfide chain and the corresponding association constants K_{β5} = 181 ± 4 M⁻¹ and K_{α5} = 136 ± 4 M⁻¹ for Na₂S₅ and Na₂S₆, respectively, with β-cyclodextrin. K_{β3} could not be estimated with as high reliability (152 ± 14 M⁻¹) as the previous ones because of the uncertainties of the small H₃ chemical shifts measured. On the other hand, considering H₄ shifts validates the correlation hypothesis (Supplementary Fig. 9).
This observation is consistent with the similar trend obtained by calculations (Fig. 3b), performed by docking the different polysulfides both on the cyclodextrin structures extracted from the Protein Data Bank (PDB) and on the ones generated by Molecular Dynamics simulations, which may better represent the cyclodextrin behavior in solution (Supplementary Figs. 7 and 8). In all cases, the general trend is a (1:1) complex formation, with \((S_n)_2^{2-}\) inside the cyclodextrin cavity (Fig. 3c). The calculated affinity scores increase with the length of the sulfide chain and thus confirm a weaker affinity for the \(Na_2S_3/\beta\)-cyclodextrin complex, explaining the experimental noisy curve for this pair (Fig. 3a, green points). Therefore, the results indicate a non-specific complex formation but highlighted a polysulfide's size-dependent complexation towards \(\beta\)-cyclodextrin.

**Fig. 2** Influence of the nature of cyclodextrin on the \(Na_2S_5/cyclodextrin\) host-guest interaction. a Experimental Job’s plot obtained following \(H_3\) shift for \(\alpha\)-cyclodextrins, \(\beta\)-cyclodextrins, and \(\gamma\)-cyclodextrins in presence of \(Na_2S_5\) (respectively, top-down). Second order polynomial fitting whose maximum is obtained for the stoichiometric ratio \(\Sigma\) (see “Methods” section) matches with the stoichiometry of the complex. b \(1H\) NMR titration plots for the inclusion of \(Na_2S_5\) in the different cyclodextrin cavities and fitted binding isotherms determined from \(H_3\) signal for \(\alpha\)-cyclodextrins, \(\beta\)-cyclodextrins, \(\gamma\)-cyclodextrin with indication of the resulting association constants \(K_5\), \(K_6\), and \(K_7\) for the corresponding 1:1 inclusion complex. The statistic error relative to the chemical shift measurement was obtained by the calculation of the standard deviation of 10 similar and independent points and is estimated to 9%. The plotted error bars were calculated considering the uncertainties propagation (see “Methods” section).

**Fig. 3** Influence of polysulfides chain length on \(\beta\)-cyclodextrin complexation. a Experimental binding isotherms for \(\beta\)-cyclodextrin complexes with \(Na_2S_5\) (red), \(Na_2S_4\) (orange), \(Na_2S_3\) (green), (See “Methods” section). The statistic error relative to the chemical shift measurement was obtained by the calculation of the standard deviation of 10 similar and independent points and is estimated to 9%. The plotted error bars were calculated considering the uncertainties propagation (see “Methods” section). b Best docking affinity scores (kcal mol\(^{-1}\)) over all the calculated \(\beta\)-cyclodextrin inclusion complexes with \(Na_2S_5\) (red), \(Na_2S_4\) (orange), and \(Na_2S_3\) (green). The first set of calculations used the cyclodextrin structures extracted from PDB as receptors (empty bars) and the second set used the cyclodextrin structures generated by Molecular Dynamics simulations (full bars) (See “Methods” section and Supplementary Fig. 4). c Corresponding best docking poses for (i) \(Na_2S_5/\beta\)-cyclodextrin, (ii) \(Na_2S_4/\beta\)-cyclodextrin, and (iii) \(Na_2S_3/\beta\)-cyclodextrin complexes. Receptors structures are those obtained from Molecular Dynamics simulations, top and side view; \(\beta\)-cyclodextrin in sticks, polysulfides in yellow space-fill representation, figure made with VMD.
experiments performed with α-cyclodextrin exhibit the same trend (Supplementary Fig. 10). Thus, the number of sulfur atoms is supposed to play a major role in the host-guest interaction. Altogether, these experiments gather essential parameters pertaining to the β-cyclodextrin interactions with polysulfides that turn out to be the key to successfully implementing our nanopore sensing technique.

Detection of the different polysulfides species by nanopore. In order to fine-tune the characterization of Na2Sx/β-cyclodextrin complex, we used the nanopore approach to detect and discriminate at the single molecule level each species. With this approach, two compartments (cis and trans) immersed with an electrolyte are separated by a protein channel, α-hemolysin (α-HL), inserted into a lipid bilayer (Fig. 4a). Applying a constant potential difference between the two electrodes in absence of any species enables the measurement of a stable pA ionic current potential difference between the two electrodes in absence of any. These current blockades are characteristic of the species size, sequence and chemical modifications. In the last years, β-cyclodextrins have been used to narrow the α-HL pore constriction to enhance the capability of the pore to discriminate nucleotides and organic compounds. For protein pores such as α-HL, the ion selectivity of the channel creates an electro-osmotic flux (EOF). The EOF is the net water flow and it is associated to the ion selectivity of the pore. Indeed, the selectivity results in an unbalance between positive and negative ion fluxes that gives rise to a net motion of water molecules. The EOF is known to affect the entrance of neutral cyclodextrin through the stem of the nanopore and its lodging into the channel. The interaction of β-cyclodextrin with α-HL has been studied as a function of a wide range of pH (5 to 11), transmembrane potentials (mV) and nature of electrolyte salt, in order to control the entry and dwell times of cyclodextrins into the nanopore. We used this strategy to compare β-cyclodextrin and Na2Sx/β-cyclodextrin complex using an α-HL pore. We first tested the effect of pH 10 on the α-HL pore (Supplementary Fig. 11) and found similar currents as previously published. The solution pH alters the protonation state of exposed amino acids and, hence, it can affect ion selectivity. A previous study indicates that α-HL selectivity inverts between pH 7.5 (anion selective) and pH 11 (cation selective). Here, we work at pH 10 where, to the best of our knowledge, no literature data provide information on the α-HL selectivity and on the direction of the EOF. In order to characterize the EOF, we hence performed a computational analysis via all-atom Molecular Dynamics simulation. We considered two different plausible titration states of α-HL at pH 10, a first one where only the

![Fig. 4 Discrimination of Na2Sx/β-cyclodextrin complex versus β-cyclodextrin using an α-hemolysin (α-HL) nanopore. a Schematic of the ion–current measurement set-up. One protein nanopore is inserted into a suspended lipid bilayer. An electrical potential is applied via two Ag/AgCl electrodes, which induces an ionic current of Na+ and Cl− ions through the nanopore (1 M NaCl, 25 mM NaHCO3, pH 10, under argon atmosphere). At pH 10, the α-HL is anion selective (see Supplementary Fig. 15), consequently, an electro-osmotic flux (EOF) directed from trans to cis sets in favoring the entry of β-cyclodextrin and Na2Sx/β-cyclodextrin into the stem part of the α-HL. b Detail of a part of current trace blockades arising from β-cyclodextrin (blue) and from Na2Sx/β-cyclodextrin complex (red) interaction with the pore at −100 mV. I0 is the ionic open pore current and Ib the blockade current. c Scatter plots of blockade ratio, given by the relation (Ib − I0)/I0, versus dwell time in presence of 1 mM β-cyclodextrin (blue, 225 events) and 1 mM Na2Sx/β-cyclodextrin complex (gray, 761 events) (5 independent experiments). d General distribution showing two populations attributed to β-cyclodextrin (blue, 64.0 ± 2.0%) and Na2Sx/β-cyclodextrin complex (red, 74.0 ± 0.3%).](https://doi.org/10.1038/s43246-020-00056-4)
seven N-Terminal (Ala1) are protonated and a second one where, in addition to Ala1, we also changed one (out of seven) Lys8 and one (out of seven) Tyr102, (Supplementary Fig. 12). For both cases, we explored transmembrane electric potentials ranging from $-500$ mV to $500$ mV. We found that, in the latter case, the anion (Cl$^-$) flow is much larger than the cation (Na$^+$) flow and the electro-osmotic flux is directed as the anions (Supplementary Figs. 13 and 14). These results suggested us to perform experiments at a negative applied voltage so that the direction of negative ions and of the EOF is trans-to-cis, see Fig. 4a. In this condition, after addition of 1 mM $\beta$-cyclodextrin or Na$_2$S$_5$/$\beta$-cyclodextrin complex into the trans compartment, we detected partial transient ionic current blockades (Fig. 5c, d) due to interactions of the molecules within the $\alpha$-HL stem. It implies that ions, here Na$^+$ and Cl$^-$ are still transported through the Na$_2$S$_5$/$\beta$-cyclodextrin complex lodged in the $\alpha$-HL while the complex cannot go through due to steric hindrance. In addition, we do not expect the polysulfides to cross the membrane via $\beta$-cyclodextrin constriction as well. This is based on the size of the (S$_n$)$_2$ species$^{51}$, $\sim 9.1$ Å (S$_3$)$_2$ or $\sim 11.7$ Å (S$_5$)$_2$, which is greater than the narrower diameter ($\sim 7.8$ Å) of the cone-like shape of cyclodextrin$^{52}$. Nevertheless, when $\beta$-cyclodextrin or Na$_2$S$_5$/$\beta$-cyclodextrin complex is added to the cis compartment, no such current blockades were observed (data not shown). This result is indicative of an EOF from trans to cis compartment preventing molecules to enter the $\alpha$-HL.

The blockade ratio, given by the relation $(I_e - I_b)/I_e$ where $I_e$ is the ionic open pore current and $I_b$ is the blockade current, versus individual event dwell times scatter plots (Fig. 4c) shows two distinct event populations for free $\beta$-cyclodextrin molecules compared to Na$_2$S$_x$/$\beta$-cyclodextrin complex (Supplementary Fig. 11), we found that the general distribution of current blockade ratios for Na$_2$S$_x$/$\beta$-cyclodextrin complex can be deconvoluted (Fig. 4d) and shows two populations centered in 74.0 ± 0.3% and 64 ± 2%. These populations were attributed to the...
Na$_2$S$_5$/β-cyclodextrin complex (74.0 ± 0.3%) and the free β-cyclodextrin in solution (64 ± 2%), respectively. The ratio between number of events associated to the Na$_2$S$_x$/β-cyclodextrin complex (red distribution) and the total number of events (gray distribution) is 81 ± 2.5%. This is in very good agreement with the NMR calculated complexation fraction in the same conditions, namely 79 ± 4% (Supplementary Fig. 16).

We then characterized Na$_2$S$_x$/β-cyclodextrin, Na$_2$S$_3$/β-cyclodextrin, Na$_2$S$_2$/β-cyclodextrin complexes and compared our results to free β-cyclodextrin molecules (Fig. 5). The current traces of each species show two kinds of current blockades associated with two types of events: (i) “bumping” events, characterized by brief, low-level current blockades, which arise due to diffusion of molecules close to the pore; and (ii) interaction events, characterized by larger current blockades of longer duration. In order to characterize the interaction of β-cyclodextrin and Na$_2$S$_x$/β-cyclodextrin complex with α-HL, each current trace was statistically analyzed to determine the event current amplitude and to separate bumping events from interaction events (see “Methods” section, the reported data refer only to interaction events). We can observe a statistical decrease in the event current amplitudes correlated to a decrease of sulfur number for each complex (Fig. 5a). We find 74.0 ± 0.3%, 68.8 ± 0.1%, 66.7 ± 0.2% for Na$_2$S$_x$/β-cyclodextrin, Na$_2$S$_3$/β-cyclodextrin and Na$_2$S$_2$/β-cyclodextrin complexes, respectively. Therefore, each complex is well discriminated from free β-cyclodextrin in solution with an average blockade ratio of 61.8 ± 0.3% (Fig. 5b).

These results strongly suggest that polysulfide species are docked inside the β-cyclodextrin cavity and that we can discriminate each species at a single sulfur atom level. In order to support this finding, we also calculated from atomistic modeling the nanopore hindrance estimator introduced as a previous publication$^{33}$. The calculation is based on an approximated quasi-1D continuum description of the nanopore electric resistance. As a first step, we calculated from Molecular Dynamics simulations for each pore section the effective area available to the electrolyte passage for both the open α-HL and the α-HL with the β-cyclodextrin in the pore stem (Fig. 5c). Then, we subtracted from the effective area of the β-cyclodextrin case a section corresponding to the occupancy of the different sulfides. This allowed us to calculate the nanopore hindrance estimator as $b = 1 - R_b/R$ where $R_b$ and $R$ are the quasi-1D resistance estimators for open pore and clogged pore. As already discussed$^{33}$, this method does not provide a quantitative estimation of the current blockade. Indeed, it is based on several simplified assumptions not satisfied at the nanoscale, the main being: (i) it is a continuum method where atomistic details enter only via the effective area profile, (ii) it is a quasi-1D method that, in principle, is valid only for smoothly varying pore section and (iii) it neglects electrical double layer effects. Nevertheless, it allows to catch the qualitative trends of the current blockades as shown by the good correlation with the experimentally observed average current blocked (see Fig. 5e and Supplementary Fig. 17).

Discussion
Via the use of a protein nanopore sensor inserted into a lipid membrane, we have demonstrated the feasibility to discriminate molecules that solely differ by a single sulfur atom. Indeed, the different polysulfide/β-cyclodextrin complexes can be identified with great sensitivity by a unique pattern of events in term of current blockade that increases with increasing the number of sulfur atoms, as intuitively expected. In addition, we could rationalize such an experimental work by molecular docking calculations and Molecular Dynamics simulations that provide evidence for the binding of the polysulfide inside the β-cyclodextrin cavity, as well as for the selective capture of the polysulfide/β-cyclodextrin complexes by the α-HL nanopore. In the present process, the polysulfide/β-cyclodextrin complexes formed in solution are specifically blocked by a nanopore embedded in a lipid membrane.

An extension of this work with respect to the Li–S technology is imminent and it enlists both the exploitation of cyclodextrin molecular recognition and its thermo-responsiveness$^9$ for enhancing battery performances (Supplementary Figs. 18 and 19). To our knowledge, stimulus-responsive$^{10}$ host-guest interaction properties have never been envisioned in batteries and we believe the described polysulfide/cyclodextrin interaction may offer some opportunities. At this stage with respect to practicality, we investigated the thermal stability of polysulfides/cyclodextrin complexes by $^1$H NMR experiments rising the temperature (Supplementary Fig. 20) and through in-situ temperature cycling experiments (Fig. 6a). We found that the complexes can dissociate and form with a fast kinetic over a narrow temperature range in a neatly reversible way by increasing and decreasing the temperature, respectively. This suggests that temperature can be used as a stimulus to regulate the capture and release of...
polysulphide species by cyclodextrins (Fig. 6). An envisioned scenario, as pictured in Fig. 6b, could be a separator membrane hosting cyclodextrins that could regulate polysulphides on demand. According to this scenario the diffusion of polysulphides intermediates towards the Li anode would be prevented while letting them participate in the redox process by thermal regeneration of the membrane.

Besides, in light of these results, the nanopore technology stands as being a powerful sensing tool to probe at the molecular level the sulfur speciation in aqueous Na-ion/polysulphide media and to test the hypothesis of manufacturing such membrane proposed in Fig. 6. Hence it may be envisioned as a novel in-situ probe of the unwanted polysulphide shuttle effect with a reliable identification of the intermediate species. However, we must admit that implementing our developed methodology to discriminate polysulphides in organic solvent would not be straightforward. It will call first for a thorough study of the polysulphide/cyclodextrin complex equilibrium in organic electrolytes that differs from the one in water, because of the different dielectric constants between both media. Moreover, as non-aqueous electrolytes are not ideal for biorelated nanopores and lipid membranes, an alternative way to conduct nanopore sensing could consist, as being planned, in considering solid-state nanopores made of polymers or semiconductors mimicking biological ionic channels such as the α-HL. Such tailored membranes will offer a nice playground for molecule discrimination near real battery conditions because of their chemical stability in organic solvents. Independently of the approach to be pursued, it remains that this nanopore technique could provide an extra analytical tool to the battery community for discriminating at the molecular level, any parasitic redox products diffusing in the electrolyte. Bearing in mind that the formation of such products can poison the positive electrode, an obvious extension of this work regards the discrimination of the solvable alkyl carbonates species well known as troublemakers in some battery systems.

In summary, a comprehensive multidisciplinary approach combining spectroscopic and electrophysiological experiments was adopted to understand deeply and at the molecular level the host-guest complexation equilibrium between the cyclodextrins and polysulphides and this was complemented by Molecular Dynamics simulations and Docking calculations. We have used this rational strategy to enable single-molecule nanopore detection, and demonstrated this technique as being a powerful tool to discriminate in aqueous medium different polysulphides with a single sulfur atom resolution, hence specifically sequencing species migrating through a membrane. That perfectly fits with the need for increasing the selectivity of separators, which could completely prevent cross communication between two electrodes in various battery technologies. Finally, the thermally controlled polysulphide/cyclodextrin complex equilibrium offers opportunities to synthesize stimuli-responsive cyclodextrin materials for the development of novel regenerative separators. Although at its early stage and being aware that further developments are needed, we hope this work to pave the way towards smarter batteries with novel regenerative and sensing separators.

Methods
All materials were used as received without further purification steps.

Preparation of sodium polysulphide solutions. Sodium polysulphide solutions (100 mM) were prepared according to a previously described method. In brief, they are formed by adding Na2S and S (purchased from Sigma-Aldrich) in stoichiometric ratios to a degassed aqueous solution buffered with 25 mM NaHCO3 and set to pH 10 under continuous stirring for a week. All the preparations are done in an Argon filled glovebox.

The characterization of the different polysulphide solutions was performed by UV-vis absorbance spectroscopy through the calculation of the different molar extinction coefficients. Absorbance spectral profiles were measured over 200–1100 nm in a 1 cm airight quartz cuvette for different diluted polysulphide solutions (0.3 mM to 1 mM) with a UVs Rio Mettler Toledo spectrophotometer (Supplementary Fig. 1).

NMR titration experiments: polysulphide/cyclodextrin complexation. Both continuous variation method and titration experiments were performed as previously described. Association constants (Kd) for the inclusion polysulphide/cyclodextrin complexes were determined in sodium bicarbonate deuterated buffer D2O (25 mM NaHCO3, pH 10) at 298 K by measuring the chemical shift variations in the 1H NMR spectra (600 MHz) of a solution of the native cyclodextrin (10 mM) in the absence and in the presence of increasing amounts of polysulphides. Polysulphides solutions were prepared as previously mentioned but in a deuterated buffer. Different diluted solutions of polysulphides (from 10 mM to 100 mM) were mixed with pre-weighted cyclodextrin (±1 mg) under Argon atmosphere. It is critically important to maintain a constant host concentration, pH and ionic strength throughout the titration experiment. Mixtures were vortexed for 1 min before acquisition of the 1H NMR spectrum on a 600 MHz Bruker spectrometer.

The chemical shifts of selected host protons resonances obtained at approximately 10 different host-guest concentration ratios with Top Spin 3.6 were plotted against the concentration of polysulphide. An iterative least-squares fitting procedure using a 1:1 stoichiometry binding model was performed with Igor Pro 6.37 to estimate for each polysulphide/cyclodextrin complex the association constant (Kd) and the maximum chemical shift (Δμmax) which is the chemical shift recorded if 100% complexation is achieved.

The one-to-one stoichiometry of the polysulphide/cyclodextrin complexes was determined using continuous method, also known as Job’s plot. The mole fraction of polysulphide was varied while keeping the total concentration of the cyclodextrin and polysulphide constant, respectively, 50 mM, 16 mM, and 50 mM for α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrins. The chemical shifts of selected host protons (Hx, essentially resonances were obtained at approximately 8 different host-guest concentration ratios with Top Spin 3.6 and were plotted against the stoichiometric ratio:

\[
\Sigma = \frac{[CD]}{[Na2Sx] + [CD]} \quad \text{(2)}
\]

According to the method, a second order polynomial fit was performed, and the stoichiometry of the studied complex was obtained from the x-coordinate at the maximum of the plot for which the concentration of the complexed cyclodextrin population is the highest.

The statistic error relative to the chemical shift measurement was obtained by the calculation of the standard deviation of 10 similar and independent points and is estimated to 9. The plotted error bars were calculated considering the uncertainties propagation assuming the experimental errors (dilution and weighting) for each condition.

Electrical detection, data acquisition, and analysis. Membrane lipid bilayers were made according to previously described methods. In brief, a film of a 1 mm solution of diphytanoyl-phosphatidylcholine-lecithin (Avanti) in anhydrous ethanol (Sigma) was spread across a 150 μm diameter hole drilled in a polysulfone wall separating the two compartments of a chamber. Each compartment contained 900 μL of 1 M NaCl, 25 mM NaHCO3, pH 10 in an Argon filled glovebox. After thinning of the decane film and formation of a planar lipid bilayer, a single α-hemolysin (α-HL) pore is inserted by adding monomeric α-HL (Sigma) from stock solution into the cis compartment. Insertion orientation of the α-HL pore was systematically checked to ensure transport of β-cyclodextrin or Na2Sx/β-cyclodextrin complex into the stem part of the pore. 100 μL of 10 mM β-cyclodextrin or 10 mM Na2Sx/β-cyclodextrin complex was added to the trans compartment. Each data set was collected using independent experiments and different pores.

Data were collected using Chimera Instruments VC100 at a sampling rate of 4.17 MHz and low-pass filtered at 10 kHz. Data were analyzed with a homemade macro using Igor software (WaveMetrics). The event measurements were based on a statistical analysis of the current traces: 425, 3904, 1254, and 1178 events analyzed for β-cyclodextrin, Na2Sx/β-cyclodextrin, Na2Sx/β-cyclodextrin and Na2Sx/β-cyclodextrin, respectively. The statistical analysis of the current traces has been previously described. As the blockades shorter than 100 μs are attributed to bumphings of β-cyclodextrin or complexes at the entrance of the channel, we removed them from the scatter plot to obtain the Fig. 4c. Nevertheless, in this figure, blockades could be attributed both to β-cyclodextrin or complexes. In order to discriminate both blockade types, we took the blockade ratio into account (Fig. 4d), which depends on the nature, shape of the anlyte. From the blockade ratio distribution, we removed the part attributed to the β-cyclodextrin, which was previously characterized in Fig. 3b, left (blue). Finally, we obtained the blockade ratio distribution attributed to the complexes (Fig. 3b). The center of each blockade ratio distribution was calculated from the average of results obtained from 5 different positions of the interval fits.
Atomistic simulation of the α-HL nanopore. Molecular Dynamics simulation set-up and production runs for estimating the ionic and electro-osmotic flows through the α-HL nanopore were performed as previously described. In brief, we employed the H+ server, version 3.2, to determine the probability that each titratable residue of the α-HL is protonated or not. This analysis allowed us to select two different plausible protonation states for the α-HL at pH 10, see Supplementary Figs. 12, 13, and 14 and Supplementary Note 2. For each of the α-HL protonation states, a tetrahedral simulations box containing the α-HL nanopore embedded in a lipid membrane was assembled by using protocols described in previous works. The POPC lipid membrane, the water molecules and the ions were added using VMD. The system was neutralized and solvated at 1 M NaCl and then equilibrated (T = 310 K, P = 1 atm). The equilibrated configuration was used as initial condition for non-equilibrium runs where a uniform and constant external electric field was applied perpendicularly to the lipid bilayer, $\hat{E} = E_z \hat{z}$. Each simulation was run for 100 ns and frames were saved every 20 ps. Average currents and electrophysmotic flow were calculated, similarly to previously reported, after discarding a 20 ns transient. Errors were estimated using block average protocol, with block length 5 ns. All the Molecular Dynamics simulations were performed using the NAMD software.\(^{(65)}\) The CHARMM36 force field\(^{(66)}\) was employed for the lipids membrane and the α-HL nanopore, while TIP3P model was used for water.\(^{(67)}\) CUFIX corrections were applied to ions.\(^{(68)}\)

**Molecular Docking of polysulfides with cyclodextrins.** To provide molecular information on the association of the inclusion complexes formed by polysulfides $S_3$, $S_4$, and cyclodextrins, we performed a series of Molecular Docking calculations. In a first set of calculations we used as receptors all the cyclodextrins crystallographic structures extracted from the Protein Data Bank, as described in Supplementary Note 1. In a second set, we used as receptors the structures generated by Molecular Dynamics simulations. The structures of the polysulfides ligands $S_3$, $S_4$, $S_5$ were extracted from crystalized proteins in the Protein Data Bank, corresponding to the query ID PSS, SAP, and S3H, respectively. All the calculations were performed using Autodock Vina.\(^{(70)}\) Ligands input files were prepared as described in Supplementary Note 1.2. The center of the box was aligned with the center of mass of the search space the search space is $20 \times 20 \times 20 \text{Å}^3$. The results for each calculation are the affinity score (kcal mol$^{-1}$) values for each ligand conformation in its respective complex.

**Data availability**

The authors declare that the main data supporting the findings of this study are available within the article and its Supporting Information file. Extra data are available from the corresponding authors on reasonable request.

**Code availability**

All MD simulation trajectories were generated using the NAMD software package \(\text{https://www.ks.uiuc.edu/Research/namd}\). Trajectory analysis are performed using the VMD software \(\text{http://www.ks.uiuc.edu/Research/vmd}\). Nanopore hindrance estimation was carried out using the method described in ref. 51. The FORTRAN code used to compute the area profiles is available at \(\text{https://github.com/giodimuccio/channelSearch}\).

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Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to J.P. or J.-M.T.

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