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A highly active and selective chalcogen bond-mediated perchlorate channel

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ABSTRACT

Artificial membrane transporters that either use chalcogen bonds to facilitate transmembrane flux of anions or show high selectivity toward perchlorate anions are rare. In this work, we report on one such novel monopeptide-based transporter system, featuring both chalcogen bonds for highly efficient anion transport and high transport selectivity toward ClO_4^- anions. Structurally, these monopeptide molecules associate with each other *via* H-bonds to produce H-bonded 1D stack that not only one dimensionally but also directionally aligns the terminal bicyclic thiophene motifs to the same side. Functionally, these well-aligned thiophenes create a sulfur-rich transmembrane pathway, combinatorially fine-tunable to enable anions to efficiently cross the membrane in the increasing activity of $Cl^- < Br^- < NO_3^- < ClO_4^-$ *via* chalcogen bonds, with EC_{50} values of 0.75, 0.40, 0.37 and 0.093 µmol/L (0.3 mol% relative to lipid molecules), respectively.

A sigma (σ)-hole bond [1–3] refers to noncovalent interactions between a lone pair of a Lewis base or an anion and an electron-deficient atom of group 14 (tetrels), 15 (pnictogens), 16 (chalcogens) and 17 (halogens) elements. Although halogen bonding has been extensively utilized to create diverse anion-binding host systems often with outstanding anion affinities and selectivities over the past ten years [1], its other sister σ-hole interactions (chalcogen, pnictogen and tetrel bonding) have received much less attention. While these σ-hole bonds have enabled ranging applications including anion sensing and catalysis [4,5], their applications to facilitate transmembrane anion transport have just begun to be explored. This is in sharp contrast to a rich family of anion transporters mediated by H-bonds [6–18]. In fact, there exist only a handful of examples using halogen [19–22], chalcogen [23,24] and pnictogen [24–26] bonds mostly as anion carriers to promote anion flux across the membrane.

In 2016, Matile and his co-workers reported 2,2′-bithiophene derivatives including **1** as the anion carrier, mediating anion transport *via* a bifurcated chalcogen bond (Fig. 1a) [23]. Among ten derivatives tested, **1** is the most active with an EC_{50} value of 1.9 µmol/L (6 mol% relative to lipid) toward chloride anions. Given that the field of anion transporters is dominated by the carriers [12,15] rather than channels [13,17,18,21] and that chalcogen bonds have been scarcely studied for ion transport [23,24], we became interested in developing anion channels making a full use of underutilized chalcogen bonds. We were further intrigued to find out the relative chalcogen bond-mediated ion-transporting activities between carrier and channel mechanisms.

In the same year of 2016, we invented a robust monopeptide-based scaffold as exemplified by **F-Phe-C4** (Fig. 1b) [27]. The H-bonding capacity and directionality of the two amide bonds provides perfect driving forces to one-dimensionally align the molecules into a 1D stack, with the same type of side chains (*e.g.*, Fmoc, Ph and C_4H_9) aligned to the same side, forming 3D gelling networks for rapid room-temperature gelation of spilled oil [27–29]. Inspired by this high directionality, we envisioned a high likelihood of generating chalcogen bond-mediated anion channel [21,30] by replacing the Fmoc groups with sulfur-based σ-holes such as **1** [23]. Conceptually, this appears to be quite straightforward. Nevertheless, given the complex structure and accordingly time-consuming synthesis of molecule **1**, there is a need for something much simpler. We therefore considered a simple commer-

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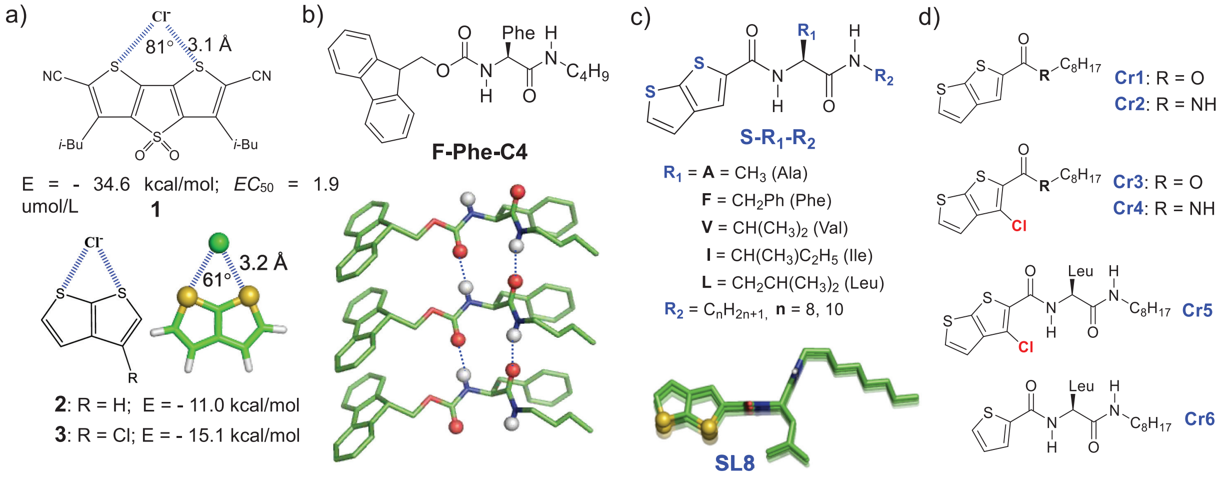

Fig. 1. (a) Bicyclic thiophene derivative **1** exploited by Matile as a chalcogen bond anion carrier [23] as well as **2** and **3** employed to construct anion channels reported in this work. (b) Crystal structure of a H-bonded 1D array made up of **F-Phe-C4** molecules. (c) The designed thiophene-containing monopeptide-based anion channels, creating a sulfur-rich transmembrane pathway for facilitating anion transport. (d) Six control compounds studied for comparison with the most active channel **SL8**.

cially available bicyclic thiophene motif **2** (Fig. 1a) as one possible alternative to **1** for developing chalcogen-bond anion transporters.

We first carried out density functional theory (DFT) computations at the M062X/6-311G** level in the gas phase to compare the bite angles, bond lengths and binding energies of 1:1 complexes involving a chloride anion and molecules **1** or **2**. Having a lengthened bond (3.2 Å), a much smaller binding energy (−11.0 kcal/mol) and a smaller bite angle (31°) than those for **1** (3.1 Å, −34.6 kcal/mol and 81°, respectively), molecule **2** likely may function as a much weaker anion transporter. Despite of this speculated weak activity by **2**, we are still inclined to believe that many of these feeble anion binders, when vertically aligned on top of each other using the monopeptide-based scaffold (*e.g.*, **SL8** in Fig. 1c), may synergistically work to achieve an effect greater than the sum of the parts. In addition, a built-in combinatorial feature of the channel scaffold allows the ion transport activity and selectivity to be quickly optimized, possibly culminating in highly active and selective anion channels.

To investigate the ion transport properties of the designed channels, we employed a pH-sensitive HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) assay (Fig. 2a) [19]. In detail, large unilamellar vesicles (LUVs) of ~120 nm in diameter (Fig. S1 in Supporting information) were prepared from EYPC lipids in the buffer (10 mmol/L HEPES, 50 mmol/L NaCl, pH 7.0), with the HPTS dye (1 mmol/L) trapped inside LUVs. A pH gradient across the membrane was created by diluting these LUV suspensions into the same type of buffer having pH 8.0. The ion transport study was initiated by adding the channel molecules into LUV suspensions and monitoring pH-dependent fluorescence emission intensities at 510 nm, with excitations at 460 and 403 nm, successfully. For the HPTS dye, the ratiometric value of I_{460}/I_{403} increases with increased pH. Using this HPTS assay, we screened a combination of 10 library members (five amino acids × two different alkyl chains, Fig. 1d). As summarized in Fig. 2b, at a concentration of 1.5 μmol/L (4.8 mol% relative to lipids), the most active channel **SL8** reaches a fractional ion transport activity of 97% after background correction (*e.g.*, 97% = (97% − 6%)/(100% − 6%)). This high activity is followed by **SL10** (63%), **SF8** (54%), **SI10** (44%), **SV10** (41%), **SF10** (40%), **SV8** (40%), **SI8** (38%), **SA10** (34%) and **SA8** (10%).

Six control molecules **Cr1**-**Cr6** were designed based on the most active **SL8** (Fig. 1d). Displaying no ion transport activity (Fig. 2c), **Cr1** clearly demonstrates that a simple bicyclic thiophene motif lacks an ability to move the ions across the membrane. Replacing the ester group in **Cr1** with an amide group generates moderately active **Cr2**. This finding, together with the highly active **SL8** that contains two amide groups, confirms the critical role played by the amide groups in linking weakly ion-binding thiophene groups *via* intermolecular H-bonds to form a transmembrane ion permeation pathway. While **Cr3**, which is structurally similar to **Cr1**, is expectedly inactive in ion transport, **Cr4** turns out to be inactive, either. These contrasting performances between **Cr2** and **Cr4** suggest the formation of a weak intramolecular H-bond between the chlorine atom and the amide H-atom, likely attenuating the ability of the amide H-atom in forming the intermolecular H-bond with the amide O-atom of another molecule. As a result, **Cr4** is incapable of H-bonding to each other to align the thiophene groups to form an ion permeation pathway. Indeed, ^{1}H NMR dilution experiments (80 – 0.62 mmol/L) performed on both **Cr2** and **Cr4** show a significant change in chemical shift by more than 0.2 ppm for the amide proton in **Cr2** and no noticeable change for that in **Cr4** (Fig. S2 in Supporting information), convincingly demonstrating the involvement of amide proton in **Cr2** in forming intermolecular H-bonds, and that in **Cr4** in forming intramolecular H-bonds. The same explanation can be used to account for why chlorine-containing **Cr5** is extremely weakly active (4%), particularly with respect to the excellent activity of 116% by the structurally similar **SL8**. But it is somewhat surprising to obtain a pretty good ion transport activity of 49% after background correction (52% before background correction) for **Cr6** that contains a monocyclic thiophene group, which again confirms the importance of amide groups in assembling multiple bicyclic thiophene groups to facilitate transmembrane ion flux.

Using the same HPTS assay in Fig. 2a but replacing NaCl salt with other alkali metal salts MCl (M = Li, K, Rb and Cs), we observed roughly similar ion transport activities that are independent of alkali metal ions (Fig. 2d). This independence suggests Cl^- anions to be the right ions transported by **SL8**, and Cl^- efflux to be accompanied by either efflux of H^+ or influx of OH^- in order to maintain the system's charge neutrality.

To compare the relative transport rates between Cl^- and H^+ or OH^-, potent proton carrier FCCP (0.25 nmol/L) was added into the extravesicular region (Fig. 2e). Under this LUV scheme, if Cl^- efflux rate is faster than H^+ efflux rate or OH^- influx rate, significant increase in fluorescence intensity of HPTS is expected. Therefore, the fact that the presence of FCCP at 0.25 nmol/L only boosts the ion transport activity of **SL8** by 7.9% (86.9%-79.0%, Fig. 2e), which is slightly larger than FCCP's intrinsic transport activity of 1.4%, indicates that although the Cl^- is transported faster than that of H^+

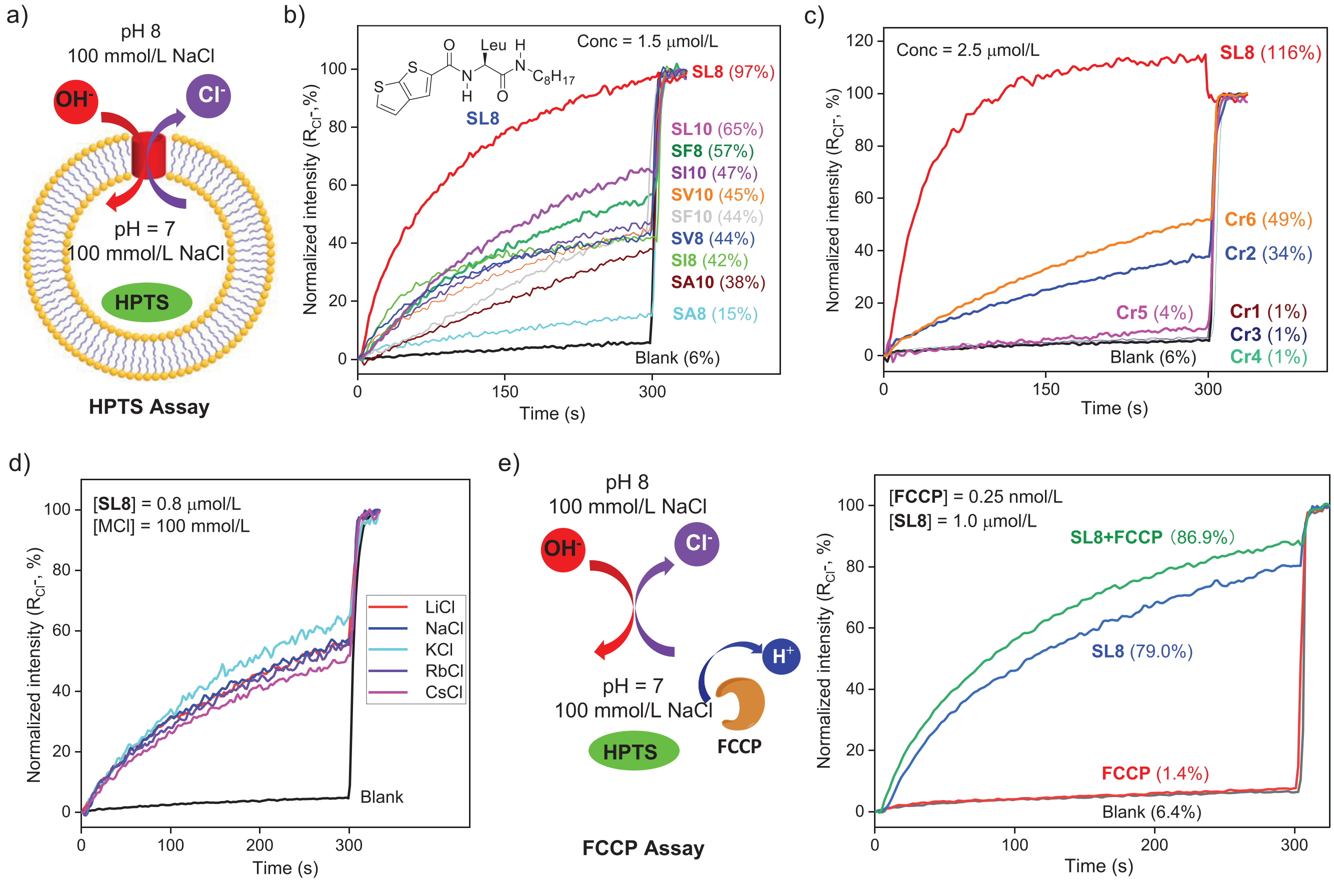

Fig. 2. (a) The pH-sensitive HPTS assay for investigating ion transport activities of the designed channels. (b) Differential ion transport activities at 1.5 μmol/L. (c) Comparative activities among **SL8** and control channels **Cr1**-**Cr6** at 2.5 μmol/L. (d) Ion transport activities obtained using HPTS assay in (a) with extravesicular region containing 100 mmol/L MCl (M = Li, Na, K, Rb and Cs). (e) The FCCP assay that applies proton carrier FCCP to elucidate the relative transport rates between Cl^- and H^+ or OH^-. To obtain the fractional ion transport activity (R_{Cl^-}), the ratiometric values of I_{460}/I_{403} at $t = 300$ s (I in the presence of channel and $I_{background}$ in the absence of channel) were first converted to percent values using equation $I\% = (I - I_0)/(I_{triton} - I_0)$, where I_0 is the value of I_{460}/I_{403} at $t = 0$ s and I_{triton} is the value of I_{460}/I_{403} at $t = 300$ s after addition of triton. This was then followed by applying equation $R_{Cl^-} = (I - I_{background})/(100 - I_{background})$, where $I_{background} = 6\%$ for (b) and (c) and 6.4% for (e). FCCP = carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone. Note that the uncorrected activity values (R) in (b, c) can be corrected using the equation of $(R - I_b)/(100\% - I_b)$ whereas background signal $I_b = 6\%$.

or OH^-, their transport rates do not differ very significantly from each other.

To clarify anion transport mechanism (carrier or channel), we have attempted to record the single channel current traces of **SL8** in symmetric baths (*cis* chamber = *trans* chamber = 1 mol/L KCl) using a Planar Lipid Bilayer Workstation (Warner Instruments), but our many attempts did fail to capture any current trace. Nevertheless, we were fortunate enough to successfully record single channel current traces (Fig. 3a) after switching from symmetric to unsymmetric baths (*cis* chamber = 1 mol/L KCl, *trans* chamber = 0.2 mol/L KCl). On the one hand, these current traces unequivocally confirm **SL8**-facilitated anion transport to proceed through a channel mechanism. On the other hand, based on the linear ohmic current-voltage (*I-V*) curve, the reverse potential value (ε_{rev}) was determined to be 31.33 mV from which the ion transport selectivity of Cl^- over K^+ was calculated to be 10 using equation $\varepsilon_{rev} = RT/F \times \ln(P_{K^+}/P_{Cl^-})$ where R = universal gas constant (8.314 J $K^{-1}mol^{-1}$), T = 300 K, F = Faraday's constant (96485 C/mol) and P = ion permeability.

Having established the channel-mediated anion transport mechanism, we applied the HPTS assay whereas both the intra- and extra-vesicular regions are filled with the same type of salts NaX (X = Cl, Br, NO_3 and ClO_4) to assess the anion transport selectivity of **SL8** (Fig. 3b). At 0.3 μmol/L, **SL8** displays increasing anion transport activities in the order of Cl^- (14%) < Br^- (33%) = NO_3^- (33%) < ClO_4^- (101%). Applying Hill analysis (Fig. 3b), further concentration-dependent measurements of transport activities yield EC_{50} value at which channel molecules reach 50% transport activity for Cl^- (0.75 ± 0.02 μmol/L). Despite the fact that motif **2** interacts with anions much weaker than **1**, channel **SL8** made up of one-dimensionally aligned **2** exhibits a chloride transport activity that is about 2.5 times as better as carrier **1**.

Additional evaluations provide EC_{50} values for Br^- (0.40 ± 0.02 μmol/L), NO_3^- (0.37 ± 0.03 μmol/L) and ClO_4^- (0.093 ± 0.004 μmol/L) (Fig. 3b and Fig. S3 in Supporting information). In the case of ClO_4^-, the corresponding EC_{50} value in terms of molar ratio relative to lipid molecules is 0.3 mol%. Based on the hydrophobic membrane thickness of 28 Å for EYPC (egg yolk L-α-phosphatidylcholine) lipid membrane [31] and an inter-chain distance of 4.8 Å in **SL8**, roughly six molecules are required to span the hydrophobic membrane region. That is, channel $(\mathbf{SL8})_6$ should represent the most likely ensembles for facilitating transmembrane anion transport. Accordingly, the true ClO_4^- transport activity of $(\mathbf{SL8})_6$ is in fact 5 times better, having an EC_{50} value of 0.05 μmol/L in terms of effective channel concentration. It might be worth mentioning that while the fluorescence emission curve excited at 460 nm looks abnormal and ion transport efficiencies therefore can't be confidently determined for F^- ions, the blank signal in the absence of channel molecules already exceeds 100% for I^- ions,

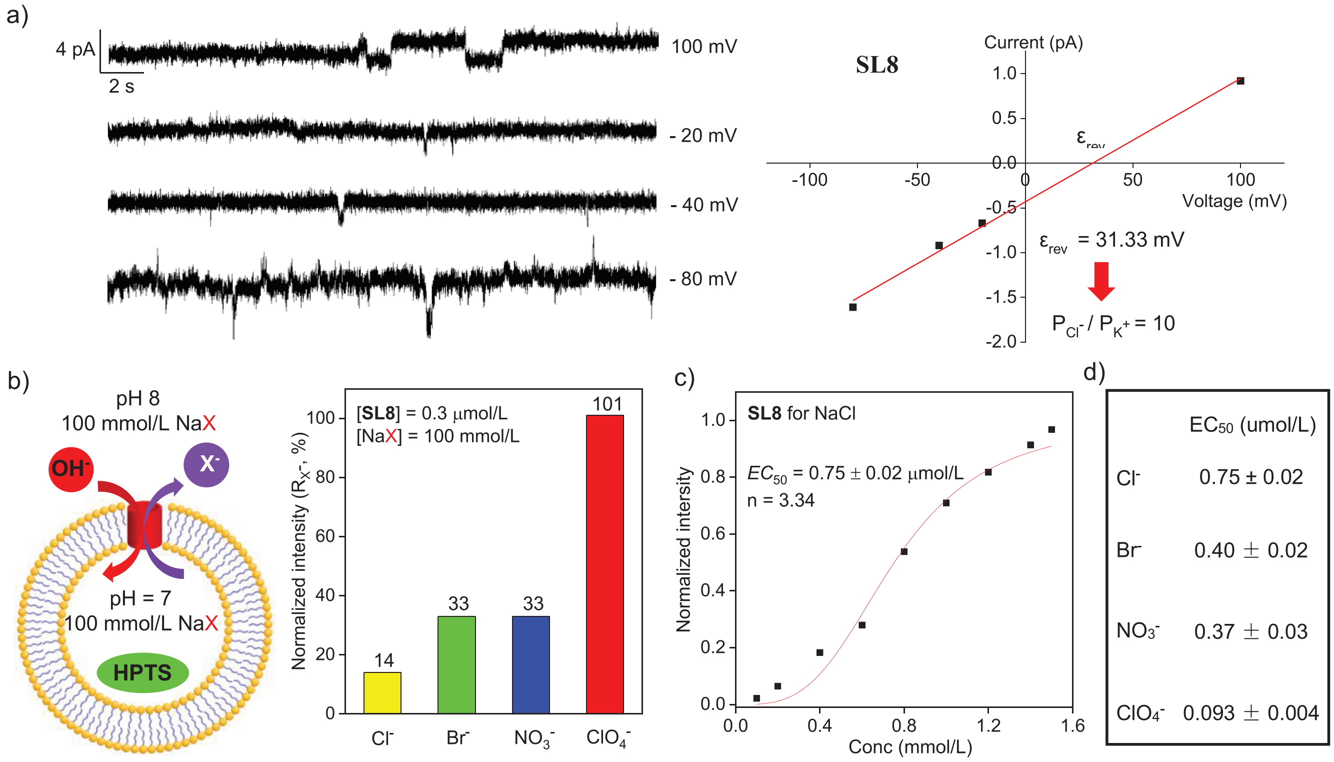

Fig. 3. (a) Single current trances recorded from −80 mV to 100 mV in unsymmetric baths (*cis* chamber = 1 mol/L KCl, *trans* chamber = 0.2 mol/L KCl) for **SL8** as well as current-voltage (*I-V*) curve for determining Cl^-/K^- selectivity. (b) The HPTS assay for assessing anion transport selectivity and for obtaining EC_{50} values for various anions.

making determination of channel-mediated I^- transport efficiency unrealistic (Fig. S4 in Supporting information).

Lastly, while artificial anion transporters have predominantly focused on the chloride transport due to their relevance to cystic fibrosis [12], their anion transport selectivity often has not been elucidated in detail. Among those with anion transport selectivity systematically investigated to include ClO_4^- anion, we are aware of transporters selective toward Cl^- [19,21,23,32], NO_3^- [33,34], I^- [31] or SCN^- [35]. To the best of our knowledge, ClO_4^--selective anion transporters are rare, and our work represents one of such rare examples and highly likely the very first example in the literature.

In summary, applying a structurally simple monopeptide scaffold recently established by us [21,27-30], we have successfully developed a chalcogen bond-mediated anion-transporting channel system. The built-in combinatorial feature of the system allows for quick optimization of membrane transport property, culminating in a discovery of a powerful yet structurally very simple anion channel **SL8**. In addition to its high transport efficiency (EC_{50} = 0.093 μmol/L or 0.3 mol% relative to lipid molecules), perchlorate-transporting channel **SL8** is unusually characterized by its high transport selectivity, having selectivity factors of 4–8 with respect to Cl^-, Br^- and NO_3^- anions. We believe our work might inspire others in the exploitation of σ-hole interactions in designing new functional materials, which is still in its infancy, for diverse applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.09.089.

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