

Research Paper

A new tool for disinfecting household drinking water for rural residents: protonated mordenite-embedded sheet

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ABSTRACT

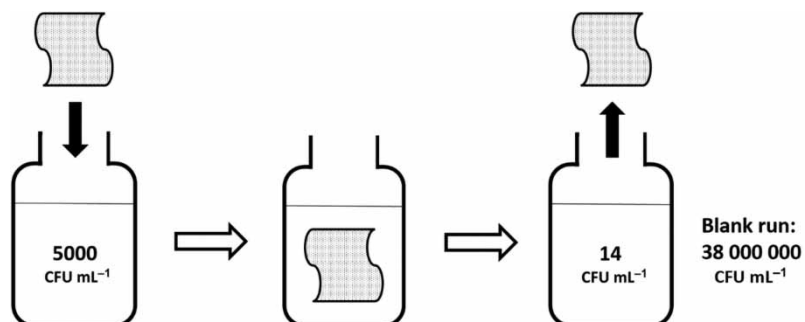
Many rural residents in developing countries drink water contaminated with feces due to inadequate purification, causing many diarrheal deaths, most of them infants. We have fabricated a protonated (H⁺-retaining) mordenite-embedded non-woven fabric sheet (H-MES) as a new tool for disinfecting drinking water at home. Proton retention amount was 1.2 mmol per gram of mordenite, 75% of its cation-exchange capacity. The H-MES released protons through cation exchange with cations in aqueous solutions, lowering the pH of the solutions to below 4. This low pH led to disinfecting 100 mL of 100-fold diluted TSB solutions containing an *Escherichia coli* species (DH5 α). For example, an initial viable count of around 5,000 CFU mL⁻¹ decreased to 14 CFU mL⁻¹ after 24 h shaking at 25 °C with added H-MES containing 0.2 g protonated mordenite; 3.8 × 10⁷ CFU mL⁻¹ without the H-MES. Adding a nitric acid solution showed a similar effect, but using chemical reagents at home might lead to unexpected accidents. Adding and removing the H-MES to and from household waterpots by hand is easy. Besides the H-MES, similar sheets embedded with bactericides and bacterial adsorbents could be a simple water-disinfection tool for rural residents.

Key words: developing countries, disinfection, drinking water, *Escherichia coli*, mordenite, rural residents

HIGHLIGHTS

- Health hazards by pathogen-contaminated water are severe among rural residents.
- A new type of bactericide, protonated mordenite, was embedded on a fabric sheet (H-MES).
- H-MES disinfected the water containing an *E. coli* species.
- Removal of H-MES from treated water is simple by hand.
- H-MES could be a simple tool for household water disinfection.

GRAPHICAL ABSTRACT



INTRODUCTION

Safe water is essential for people to wash their hands, cook, and drink, but it is not readily available in rural areas of developing countries. Harmful contaminants in the water are mainly heavy metals, arsenic, and pathogenic bacteria. The World Health Organization (WHO) reported that at least two billion people drink fecal-contaminated water containing pathogens, associated with 485,000 diarrheal deaths each year, most of them infants (WHO 2019). These health hazards posed by pathogen-contaminated water are severe, especially in rural areas of developing countries (WHO 2019); even commercial sachet water sometimes contains pathogens (Aslan *et al.* 2021).

The best way to solve this problem is to develop infrastructures that provide clean water, such as a tap water system. However, in the short term, the priority should be supplying simple and effective methods to purify water for individual use. Currently, many rural residents disinfect drinking water through flocculation, filtration, solar disinfection with and without titanium oxide, chlorination, and boiling (Pooi & Ng 2018; Bailey *et al.* 2021; Marobhe & Sabai 2021). These methods work if used properly, but the grave health hazards noted in the above-mentioned WHO report indicate that some residents are not using or are improperly using the methods. For example, many governments, municipalities, and organizations recommend chlorination with sodium hypochlorite and sodium dichloroisocyanurate. However, some people are hesitant to chlorinate because of the taste and smell of the treated water (Crider *et al.* 2018; Ali *et al.* 2021). Also, some of these disinfection methods need skills for proper use, require maintenance, and are time- and energy-consuming. Therefore, besides the current methods, there is a need for simple and cost-effective methods acceptable to rural residents.

A new type of disinfectant, zeolites containing antibacterial heavy metal cations such as Ag^+ and Cu^{2+} , disinfect water by releasing these cations into the water (Rivera-Garza *et al.* 2000; Cui *et al.* 2021). Nanoparticles of these heavy metals also disinfect water on their own besides the heavy metal cations released from the zeolites (Wypij *et al.* 2018; Cui *et al.* 2021). In these cases, however, the concentration of the heavy metals in the treated water requires attention because these heavy metals are also toxic to humans. For example, the WHO guideline value for Cu in drinking water is 2 mg L^{-1} , with 0.1 mg L^{-1} suggested for Ag, although available data was inadequate to permit derivation of a health-based guideline value (WHO 2017). For Ag, the United States Environmental Protection Agency (EPA) indicates a regulation value for secondary drinking water of 0.1 mg L^{-1} (EPA 2021).

Besides these heavy metal cations, high concentrations of protons can kill bacteria in water because many bacterial species do not survive at low pH, below around 4, except for acidophiles (Rosso *et al.* 1995; Fernández-Calviño & Bååth 2010; Qusheng & Matthew 2018). Therefore, we propose lowering the pH to below 4 to kill many kinds of bacterial pathogens in water. Adding acid solutions similarly lowers the pH, but using chemical reagents at home may not be widely recommended for safety reasons. Hence, we used a mordenite-type zeolite containing protons to lower the pH, where the zeolite releases protons into the water through cation exchange with cations in the water.

This study aimed to investigate the disinfection property of the protonated mordenite using aqueous solutions containing an *E. coli* species (DH5 α) as a model bacterium. We used mordenite as the cation exchanger because of its higher acid-resistance among zeolite species and hence little structure destruction with the protonation (Munthali *et al.* 2014). A natural mordenite, readily available and inexpensive, was used. We fabricated a non-woven fabric sheet embedded with protonated natural mordenite to facilitate the movement of the mordenite into and out of the water (Sadia *et al.* 2021). Based on the results, the potential of the embedded sheet as a new household water-disinfection tool for rural residents is discussed.

MATERIALS AND METHODS

Materials

Chemical reagents were from Nacalai Tesque, Inc., Japan. A natural mordenite sample (70 mesh) was from Nitto Funke Kogyo K.K., Japan. The *Escherichia coli* species, DH5 α Competent Cells High Efficiency (DH5 α), was from Promega Co., Japan. The K^+ -saturated mordenite was prepared by repeated washing of the natural mordenite with 1 mol L^{-1} KCl. The cation-exchange capacity (CEC) of the mordenite sample was determined by measuring the amount of K^+ extracted from the K^+ -saturated mordenite by repeated washing with 1 mol L^{-1} NH_4Cl . The metals, including K, were quantified using an atomic absorption spectrophotometer (AAS; Hitachi, Z-5000). Powder X-ray diffraction (XRD) analysis of the mordenite samples was performed using an Ultima IV (Rigaku) with $\text{CuK}\alpha$ radiation at 40 kV and 40 mA.

Fabrication of H-MES

A non-woven fabric sheet embedded with the natural mordenite powder was fabricated similarly to the methods described by Botoman *et al.* (2018) and Sadia *et al.* (2021) as follows. A commercially available water-permeable non-woven fabric sheet was cut into squares, each about 80 cm² and 0.1 g. The square fabric sheet was sandwiched with mordenite powder, gently pressed, and heated in an oven at 160 °C for 8 min. After cooling, 20 fabric sheets were put in a 1-L bottle and shaken reciprocally with 800 mL of distilled water at 150 rpm for 1 h to remove unattached mordenite powder. After repeating the shaking twice, the fabric sheet was air-dried (mordenite-embedded sheet, MES). The MES was shaken twice with 800 mL of 1 mol L⁻¹ KNO₃ at 80 rpm for 3 h to saturate their cation-exchange sites with K⁺, washed with water, and air-dried (K⁺-saturated MES, K-MES). The mass of mordenite embedded in the K-MES was measured. Fourteen pieces of the K-MES containing about 1 g of mordenite was reciprocally shaken with 200 mL of 20 mmol L⁻¹ HNO₃ in a 1-L bottle at 80 rpm for 3 h. The resulting partially protonated (H⁺-retaining) K-MES is abbreviated as H-MES. The H⁺-retention per unit mass of mordenite in the H-MES was the K⁺ released into the HNO₃ solution, assuming equal amounts of retained H⁺ and released K⁺.

Disinfection experiments

In the disinfection experiments, the tools and solutions were hydrothermally sterilized at 121 °C for 20 min, except for the solutions containing DH5 α . First, 1 mL of a stock solution of DH5 α kept in a freezer at -80 °C was mixed with 50 mL of Trypticase soy broth (TSB) solution and put in an incubator at 37 °C for 24 h. Then, 1 mL of the incubated solution was mixed with 100 mL of TSB solution and put in an incubator at 37 °C for 6 h. After diluting with TSB solution to different ratios, a 1 mL portion was mixed with 100 mL of distilled water in a 250 mL bottle to obtain the test solution. After allowing the test solution to stand for 1 h, it was shaken at 25 °C at 80 rpm with or without adding H-MES containing 0.2 g of mordenite. The shaking speed at 80 rpm is a simulation of mixing speed by hand when the sheet is applied in households. The purpose of standing was to activate DH5 α because this *E. coli* species hardly multiplied immediately after mixing with water: in a preliminary experiment, the viable count remained unchanged for 30 min but increased about 4-fold after 2 h. The change in the viable count of the test solution was determined over time by a plate dilution method using Lysogeny Broth agar medium. Adding K-MES instead of H-MES was also investigated. The disinfection experiments were conducted in triplicate.

RESULTS

Characterization of mordenite

The XRD pattern of the natural mordenite powder (Figure 1(a)) showed all the peaks of standard mordenite (IZA 2021) and a peak of quartz at 26.7°. Chemical analysis of the natural mordenite showed a Si/Al atomic ratio of 4.7, close to the standard value of 5.0 for mordenite (IZA 2021). The CEC value, the K⁺ content of K⁺-saturated mordenite, was 1.60 mmol g⁻¹, lower than that of standard mordenite, 2.29 mmol g⁻¹. The lower value indicates the coexistence of mainly amorphous impurities whose peaks are not recognizable in the XRD pattern.

Protonation behaviour of K-MES

Before fabricating the H-MES for the disinfection experiments, we added 200 mL of different concentrations of HNO₃ solution to the K-MES containing 1 g of mordenite and determined the amount of H⁺-retention of (K⁺-released from) the K-MES (Figure 2(a)). H⁺-retention increased with the amount of HNO₃ added. The data points were located approximately on the mathematically calculated curve (the dotted curve in Figure 2(a)) that assumes equal adsorption selectivity of H⁺ and K⁺ towards negative charges of mordenite. In this calculation, the amounts of negative charge and K⁺-retention of mordenite in the K-MES are 1.60 mmol g⁻¹, CEC of mordenite. For example, adding K-MES containing 1 g of mordenite to 1.60 mmol of HNO₃ gives an H⁺-retention amount of 0.80 mmol g⁻¹. Figure 2(a) also shows the data points for the K⁺-saturated mordenite powder, slightly greater but similar to those of K-MES. This result indicates that embedding mordenite particles in the fabrics had little effect on the reaction between the particles and H⁺ in the solution because each mordenite particle is embedded in the fibre exposing more than half of it (Sadia *et al.* 2021). The H-MES used for the disinfection experiments retained 1.20 mmol of H⁺ per gram of mordenite, with 75% of the cation-exchange sites retaining H⁺. Figure 1(b) shows the XRD pattern after washing 1 g of the mordenite powder with 200 mL of 20 mmol L⁻¹ HNO₃ for 3 h. The XRD

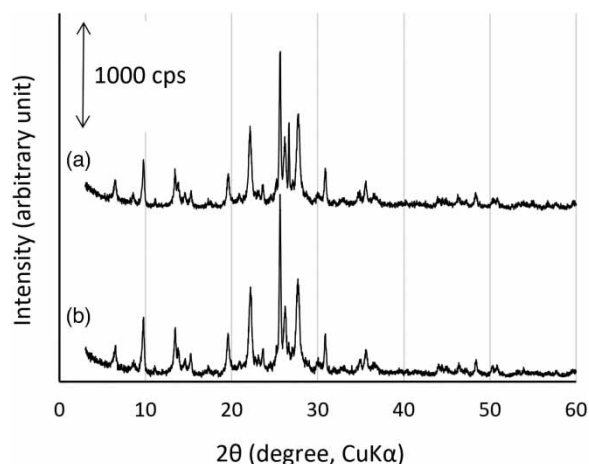


Figure 1 | Powder XRD patterns of (a) untreated natural mordenite and (b) HNO₃-treated mordenite.

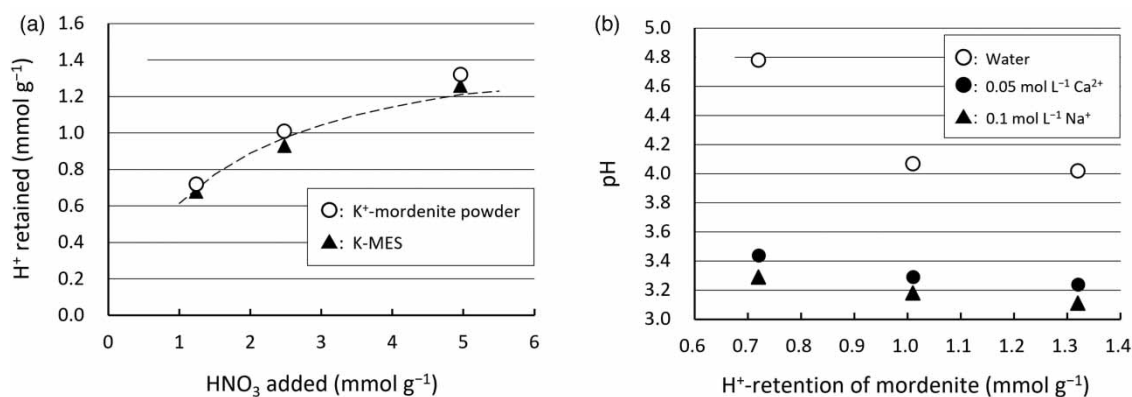


Figure 2 | Protonation and deprotonation properties of mordenite. (a) amount of H⁺ retention on the K-MES and K⁺-saturated mordenite with different amounts of added HNO₃. The dotted curve shows mathematically calculated values assuming equal adsorption selectivity of H⁺ and K⁺ towards mordenite; (b) pH of aqueous solutions with added K⁺-saturated mordenite with different amounts of H⁺ retention.

pattern was similar to that without the HNO₃-washing (Figure 1(a)), indicating few effects of the washing on the mordenite structure. Figure 1(b) did not show the peak of quartz: this is due to the sampling error of the powder between the two cases.

Proton release property of H-MES

The release of H⁺ from the protonated mordenite in the H-MES occurs mainly by cation exchange with cations in aqueous solutions. Therefore, the concentration and species of the cations in the aqueous solutions affect the decrease in pH. Thus, we shook 90 mL of distilled water and different salt solutions with 0.18 g of protonated mordenite with different H⁺ retentions and measured the pH (Figure 2(b)). The pH decreased with increasing H⁺ retention of the mordenite for all solutions. The pH was the highest for water because it contains no cations to exchange with protons on the mordenite. The pH was lower for the Na⁺ solution than the Ca²⁺ solution: Na⁺ exchanged more protons than Ca²⁺. This observation is consistent with the results that mordenite has higher adsorption selectivity for monovalent cations than divalent cations (Liang 1999; Munthali *et al.* 2015).

Disinfection property of H-MES

Shaking the test solution with a DH5 α viable count of around 5,000 CFU (colony forming unit) mL⁻¹ at 25 °C, without adding H-MES, increased the viable count over time: 1.0 \times 10⁵ CFU mL⁻¹ after 6 h; 3.8 \times 10⁷ CFU mL⁻¹ after 24 h (Figure 3(a), the blank run). This result indicates that the solution composition and temperature during the shaking process were suitable for the growth of DH5 α . The pH of the test solution was between 6.5 and 6.7, similar to the 6.6 of the TSB solution. On

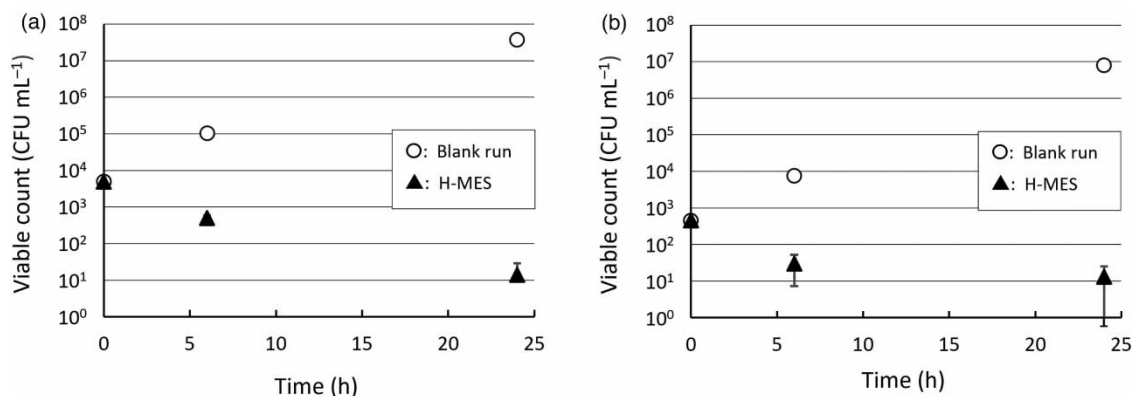


Figure 3 | Viable count of *DH5α* at different shaking times with added H-MES at an initial viable count of around (a) 5,000 CFU mL⁻¹ and (b) 500 CFU mL⁻¹. Error bars show standard deviation.

the other hand, adding K-MES embedded with about 0.2 g of mordenite to the test solution did not affect the viable count and pH at all shaking times (data not shown): the K⁺-saturated mordenite and the fabric sheet do not affect the growth of *DH5α*.

In contrast, adding H-MES embedded with 0.2 g of mordenite to the same test solution reduced the viable count over time: 500 CFU mL⁻¹ after 6 h; 14 CFU mL⁻¹ after 24 h (Figure 3(a)). The pH of the test solution decreased from 6.6 to 3.8 after 1 h and remained there until 24 h. The test solution, 100-fold diluted TSB, contained Na⁺ and K⁺ with a total concentration of 0.11 mmol L⁻¹, and the pH value was as predicted from the results in Figure 2(b): pH 3.8 was closer to that of water than 0.1 mol L⁻¹ solution of Na⁺. Adding 0.5 mL of 0.1 mol L⁻¹ HNO₃ (0.05 mmol HNO₃) to the test solution gave a pH of 3.8. Because the H-MES embedded with 0.2 g of mordenite retains 0.24 mmol of H⁺, the release of H⁺ from the H-MES was roughly estimated as 21%. The same experiment with the test solution with a lower viable count of around 500 CFU mL⁻¹ showed similar results: 30 CFU mL⁻¹ after 6 h; 13 CFU mL⁻¹ after 24 h (Figure 3(b)). The blank run was 7.5 × 10³ after 6 h, 7.9 × 10⁶ after 24 h. The pH values at all times were similar to those with a viable count of around 5,000 CFU mL⁻¹. These results indicate that the low pH caused by the release of H⁺ from the H-MES killed most of the *DH5α*, even when blank runs increased its viable count by about 10⁴ times after 24 h.

We carried out the same disinfection experiment by adding HNO₃ solution, instead of adding H-MES, to the test solution with an initial *DH5α* viable count of around 4,000 CFU mL⁻¹. Adjusting the initial pH of the test solution to 3.6 using 0.55 mL of 0.1 mol L⁻¹ HNO₃ maintained the pH until 24 h (Figure 4). The viable count decreased to 1,307 CFU mL⁻¹ after 6 h and 417 CFU mL⁻¹ after 24 h, showing the disinfection effect by adding HNO₃. The level of disinfection was somewhat lower than that of Figure 3(a), though the initial viable count, pH values, and the viable counts of the blank run were

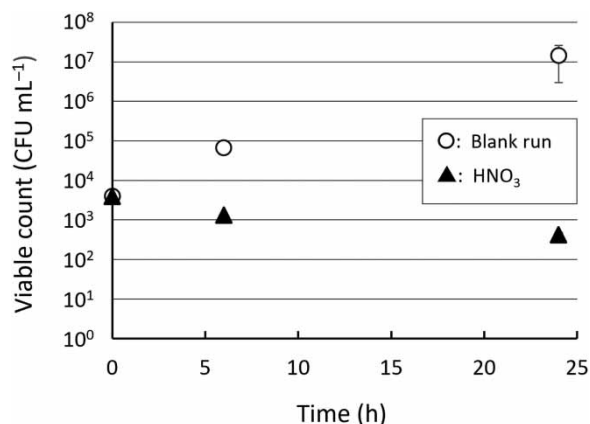


Figure 4 | Viable count of *DH5α* at different shaking times with added HNO₃ at an initial viable count of around 4,000 CFU mL⁻¹. Error bars show standard deviation.

slightly lower than those of Figure 3(a). This result indicates that H-MES disinfected the test solution not only by lowering the pH of the test solution but also by other factors, and the mechanism of which needs to be clarified in future studies.

DISCUSSION

H-MES killed almost all the DH5 α in the 100-fold diluted TSB solutions, showing the potential of H-MES as a tool for household disinfection. However, disinfection was incomplete because the viable count did not reach zero even after 24 h, meaning the water was not sufficiently safe for drinking. Disinfection was affected by reaction time. Temperature, the composition of water, and bacterial species may also affect the disinfection. Therefore, more study is needed to enhance the bactericidal ability of H-MES. Retaining Cu²⁺ together with H⁺ on H-MES is one enhancement; a preliminary experiment with a Cu²⁺-retaining H-MES increased the disinfection degree and speed with a final Cu concentration of 0.8 mg L⁻¹. The Cu²⁺ concentration should be lower than 2 mg L⁻¹ in the treated water (WHO 2017), and the Cu²⁺ concentration of the treated water is adjustable by the amount of Cu²⁺ retention of the MES.

The drinking water treated by H-MES has a low pH, below 4. Although the WHO does not suggest a health-based guideline value for the pH of drinking water (WHO 2019), some people may hesitate to drink low pH water, despite there being many acidic beverages on the market. However, our simple test showed that the pH of the treated water increased immediately to neutral or slightly alkaline by adding a sheet embedding with synthetic zeolite-A. This zeolite is saturated with Na⁺ and releases a small amount of Na⁺ into the water, adsorbs an equal amount of H⁺ from the water, and increases pH (the H⁺ comes from water molecules leaving OH⁻ in the water). Zeolite-A has higher H⁺-adsorption selectivity among zeolite species (Munthali *et al.* 2014) and raises the pH of aqueous solutions. Therefore using a zeolite-A-embedded sheet after disinfecting drinking water will solve the problem of low pH. This method is for residents who want to avoid low pH water for drinking.

Powdery protonated mordenite also disinfects water in household waterpots, but it remains in suspension, and therefore, needs to be removed by filtration or sedimentation. Embedding powder in a fabric sheet, like H-MES, is one method that gives a physical form to powder and enables the simple removal of the powder from the treated water. The sheet embedded with anion exchangers may remove anionic contaminants such as arsenate and nitrate and may also remove pathogenic bacteria and viruses because they are usually negatively charged. Anion-exchange resins and anion-exchange fibres are available, but they are expensive for our purpose. LDH, layered double hydroxide, has positive charges in its structure and could be a cost-effective adsorbent for bacteria and viruses (Kim *et al.* 2012; Forano *et al.* 2018). Giving physical form to LDH is the future target of our research because it is powdery.

CONCLUSION

We aimed to develop a simple and effective tool for disinfecting water in household waterpots of rural residents in developing countries. A natural mordenite powder was embedded in a fabric sheet and partially protonated to form H-MES, a new type of disinfectant. The H-MES released the protons, the protons lowered the pH to below 4, killing DH5 α in the aqueous solutions. The level of disinfection was high, although a few DH5 α remained in the treated water even after 24 h. However, because the goal of our study is complete disinfection, we need to improve the performance of the H-MES to attain that goal.

Adding H-MES instead of adding acids for lowering the pH of water could prevent accidents due to misuse at home, and the H-MES does not affect the smell and taste of the water like chlorination does. Also, the H-MES enables simple water disinfection in batch systems because of simple removal from the treated water. Besides protonated mordenite, other materials that kill or adsorb bacteria could easily disinfect batch water at home if given a physical form like the H-MES.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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