

Supplementary Information for:

**Probing the Hepatitis B virus e-antigen with a nanopore sensor based on collisional events analysis**

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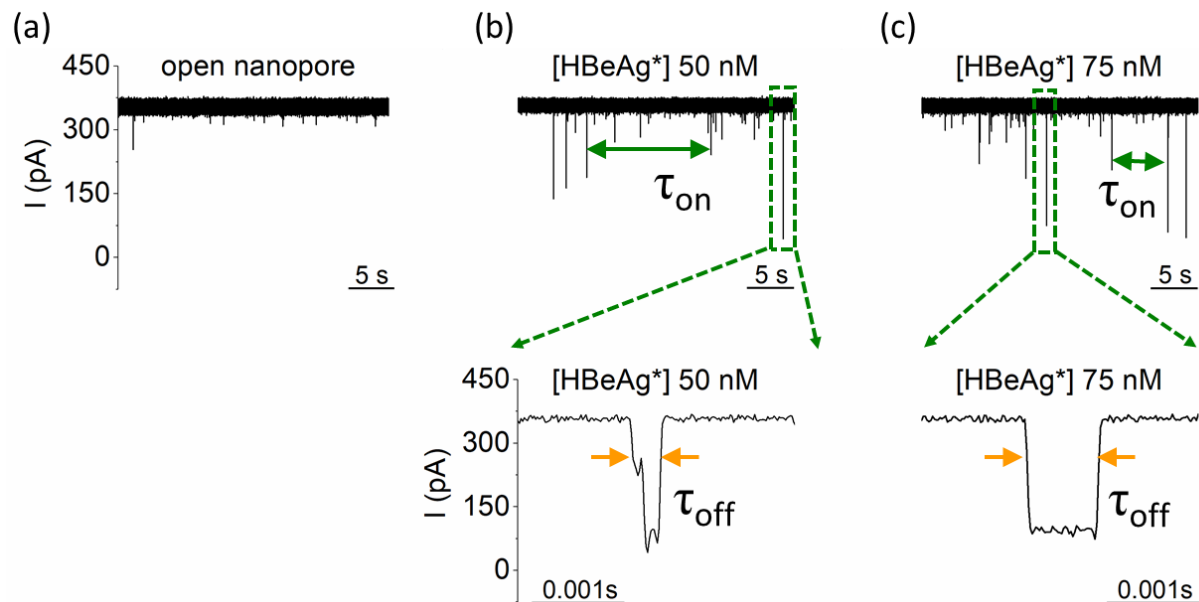


Figure S1. **Bumping interactions between the  $\alpha$ -HL nanopore and HBeAg\* are visible through electrophysiology recordings.** The ionic current through the  $\alpha$ -HL (a) gets reversibly blocked (downwardly oriented spikes) upon nanopore interactions with the *cis*-side added HBeAg\* at various concentrations of 50 nM (b) and respectively 75 nM (c). In the expanded traces we display characteristic HBeAg\*-induced blockade events. The traces were recorded at an applied potential  $\Delta V = +180\text{mV}$  in a 2M KCl electrolyte solution, buffered at pH = 8 with a 10 mM HEPES.

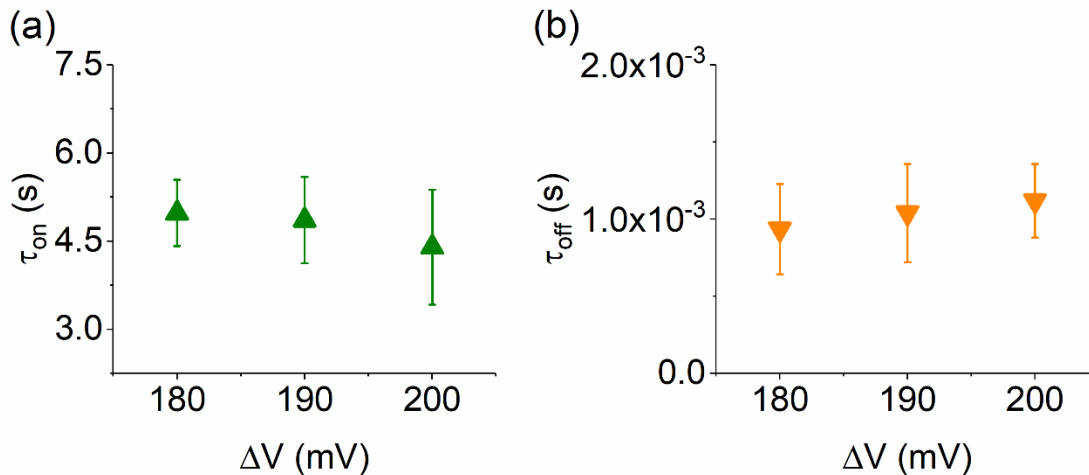


Figure S2. **Statistical analysis of  $\alpha$ -HL-HBeAg\* interactions.** (a) In accord with the expected physical scenario, the negatively charged, *cis* side-added HBeAg\* protein interacts more favorably with a single  $\alpha$ -HL nanopore at increasing positive values of the holding potential, as suggested by the small, yet consistent decrease of the inter-events average time intervals ( $\tau_{on}$ ). (b) The average dissociation time intervals ( $\tau_{off}$ ) slightly increase with the increase in the holding potential across the positively biased nanopore, suggesting the release of the HBeAg\* in the same chamber upon unbinding from the nanopore.

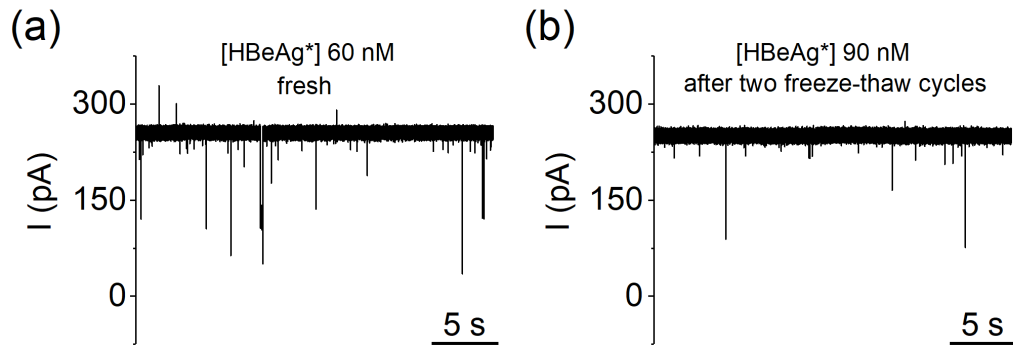


Figure S3. **HBeAg monomers dimerize in aqueous solution and are precluded from the interaction with the  $\alpha$ -HL vestibule entrance.** Ionic current blockades recorded through a single  $\alpha$ -HL at an applied voltage  $\Delta V = +130$  mV in a 2 M KCl electrolyte buffered at pH=8, induced by freshly dissolved HBeAg\* (60 nM) added on the *cis* chamber (a) and after two freeze-thaw cycles (b). The occurrence of blockade events is visibly decreased in the latter case, even at a higher concentration of the HBeAg\* (90 nM). Herein, the symbol ‘\*’ associated with the HBeAg denotes that the purchased antigen (see Materials and Methods section).

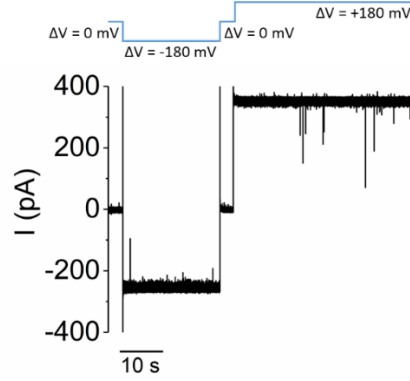


Figure S4. **The polarity of the transmembrane voltage critically sets the occurrence of monoclonal Ab(HBeAg)- $\alpha$ -HL nanopore interactions.** When present at bulk concentration of 100nM on the *cis* side of a lipid membrane isolating a single  $\alpha$ -HL nanopore, collisional events reflecting nanopore-analyte interactions were seen only at *trans*-positive potentials.

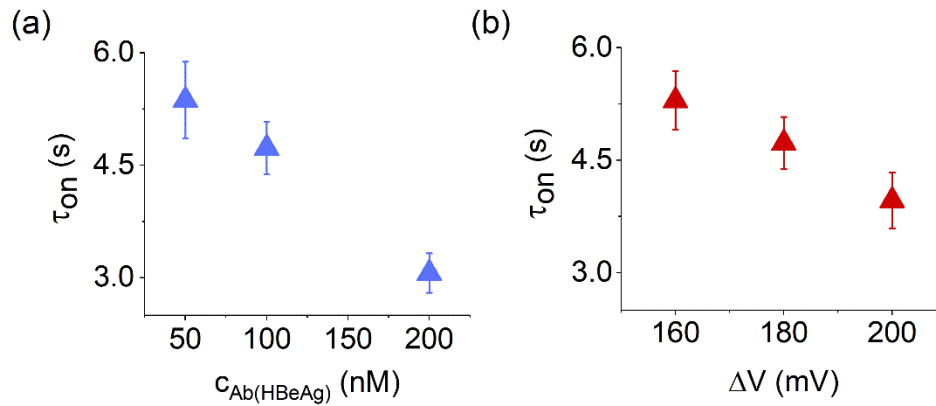


Figure S5. **The concentration- and voltage dependent association of the monoclonal Ab(HBeAg) antibody with a single  $\alpha$ -HL nanopore.** (a) The average antibody-nanopore association time ( $\tau_{on}$ ) measured at increasing nanomolar concentration of antibody extracted from recordings made at an applied voltage  $\Delta V = +180$  mV. (b) The average antibody-nanopore

association time ( $\tau_{on}$ ) measured at increasingly applied voltages, with the Ab(HBeAg) added at a bulk concentration of 100 nM.

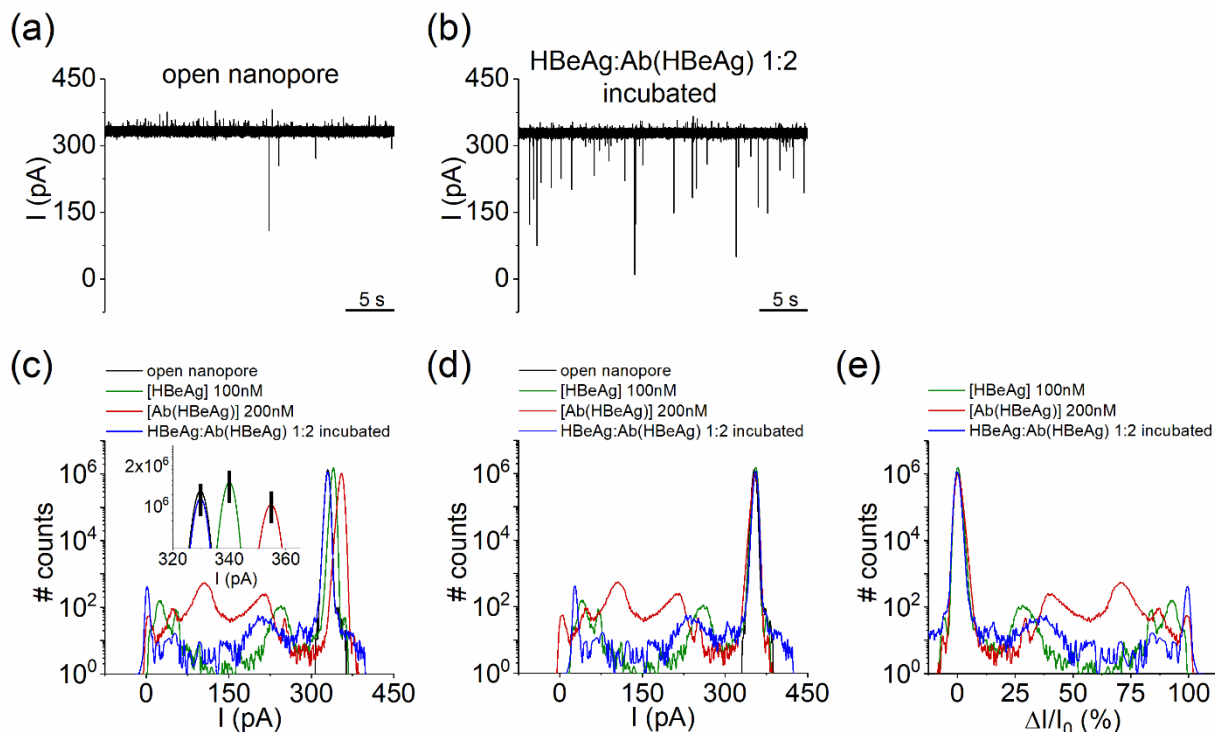


Figure S6. **Detection of pre-incubated HBeAg-Ab(HBeAg) complexes with the  $\alpha$ -HL nanopore.** (a, b) Interaction between  $\alpha$ -HL nanopore and a *cis* added mixture of HBeAg and Ab(HBeAg) (1:2 molar ratio), previously incubated for 2h 30min at room temperature. (c-e) The histograms showing the different conductive states of  $\alpha$ -HL nanopore while interacting with the HBeAg-Ab(HBeAg) mixture (blue curve), relative to those measured with either HBeAg (green curve) or Ab(HBeAg) (red curve) alone. The applied voltage in such measurements was  $\Delta V = +180$  mV, and the recording buffer was similar as above.