Supporting Information

A Possible Mechanism of Ion Selectivity in Eukaryotic Voltage-Gated Sodium Channels

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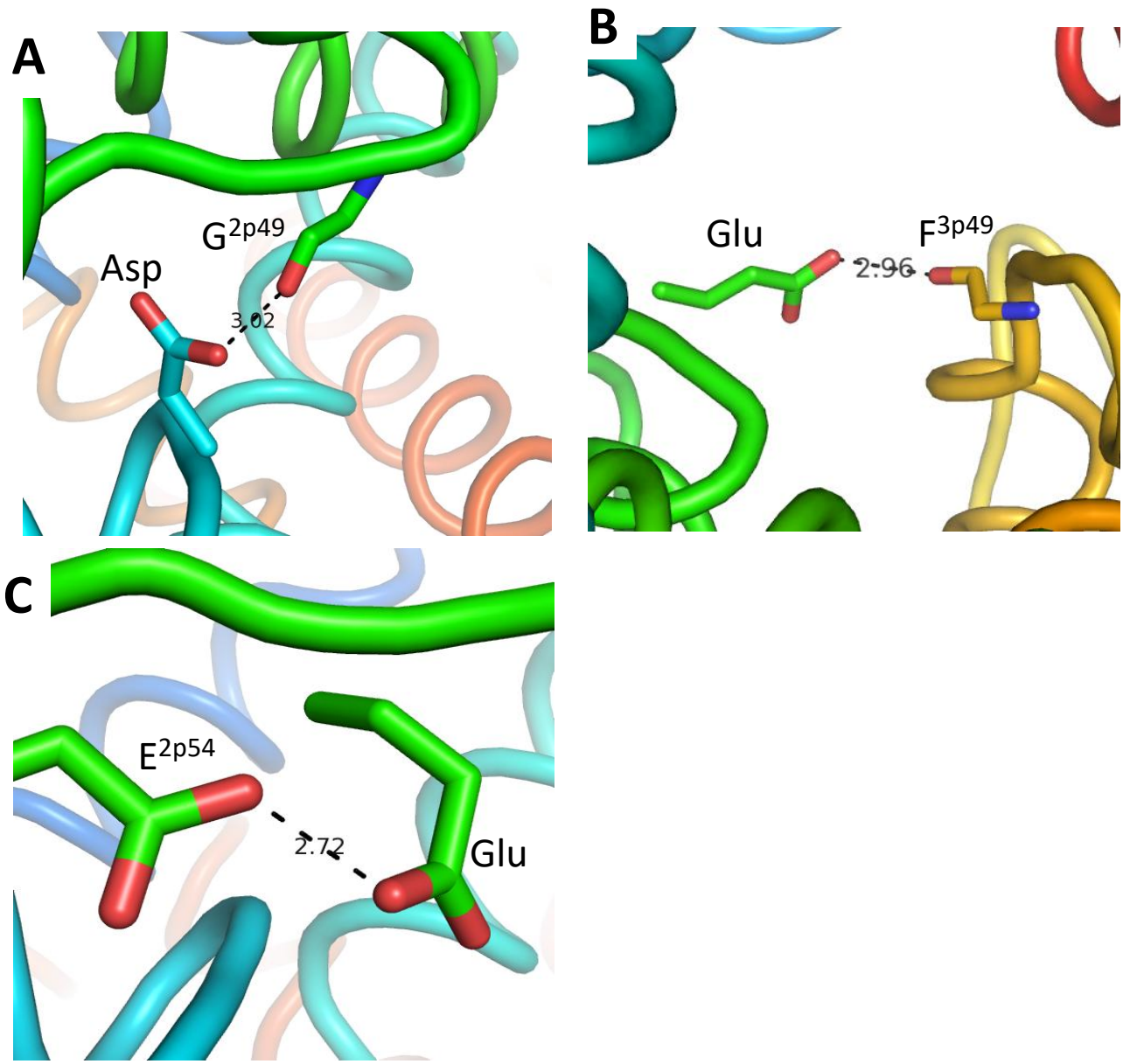


Figure S1. Cryo-EM structures of Nav channels suggest protonation of acidic residues. ***A.*** In the cryo-EM structure of NavPaS with TTX (PDB ID: 6A95), the distance between Asp and $^{O}G^{2p49}$ and mutual disposition of the C-O and O=C bonds suggest that protonated Asp donates an H-bond to G^{2p49}. ***B.*** In the cryo-EM structure of rNav1.5 with flecainide (PDB ID: 3uz0), the distance between oxygens Glu and $^{O}F^{3p49}$ and mutual disposition of the C-O and O=C bonds suggest that protonated Glu donates an H-bond to $^{O}F^{3p49}$. ***C.*** In the cryo-EM structure of Apo rNav1.5 (PDB ID: 3uz3), the distance between oxygens in Glu and E^{2p54} and mutual disposition of the C-O bonds suggests that the two acidic residues share a proton.

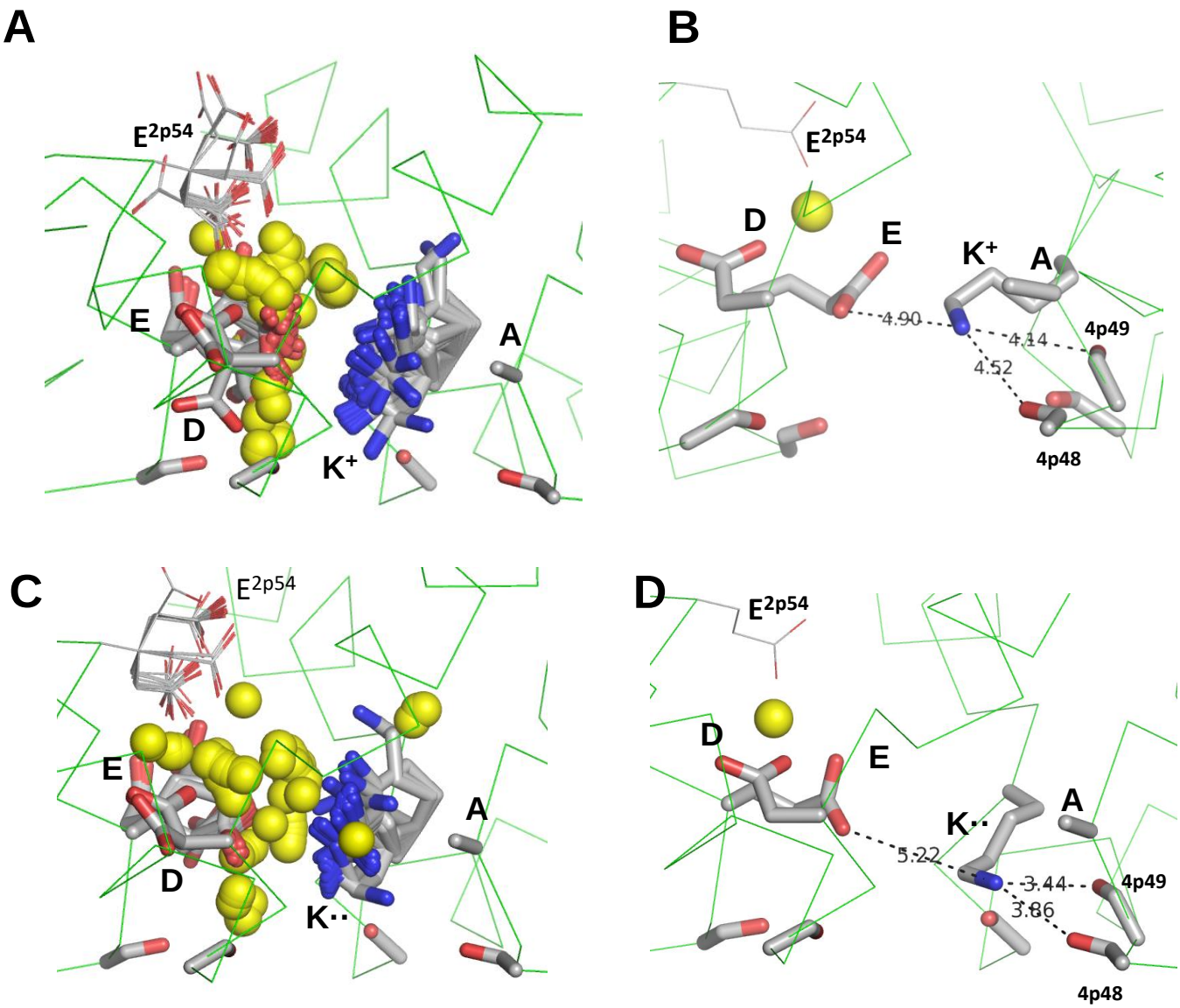
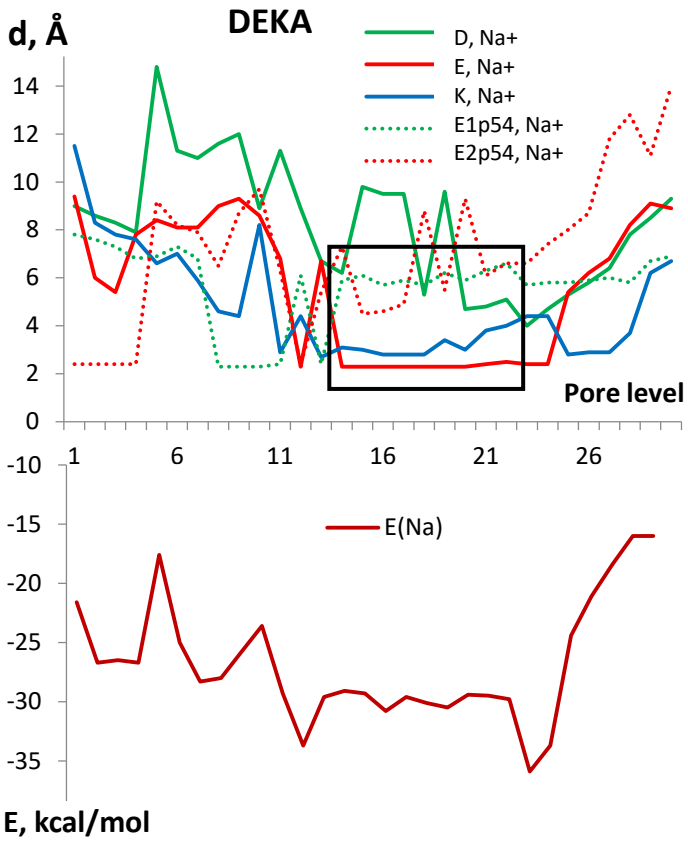


Figure S2. **MC-minimized models of Nav1.4 with Na^+. *A.*** Ensemble of MECs within 10 kcal/mol from AGM of the Nav1.4 model with Na^+ and Lys^+. Computations were performed with HotGrid (see Methods). Na^+ is chelated between carboxylates, while Lys^+ and Glu rarely form a salt-bridge. **B.** In the AGM of the ensemble (A), the ammonium group of Lys^+ is at rather large distances from Glu and backbone carbonyls. The salt bridge with Glu is not formed due to repulsion from Na^+. **C.** Ensemble of MECs within 10 kcal/mol from AGM of the Nav1.4 model with Na^+ and Lys··. **D.** AGM of the ensemble (C). See section 3.2 for more detail.



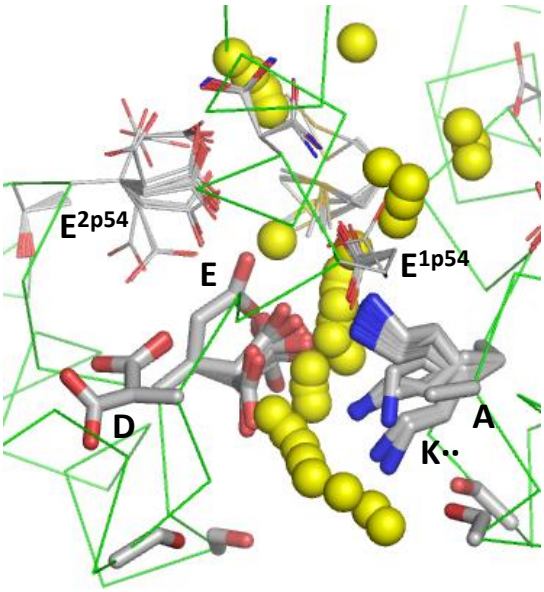


Figure S3. **Na^+ trajectory computed with dielectric function $\varepsilon=2d_{ij}$**. (d_{ij} is distance between atoms.) Na^+ is chelated between Lys·· and Glu- at levels 14-18 and 20 of the DEKA region. Lys·· adopts dunking orientation at levels 26-28 of the 8CO region.

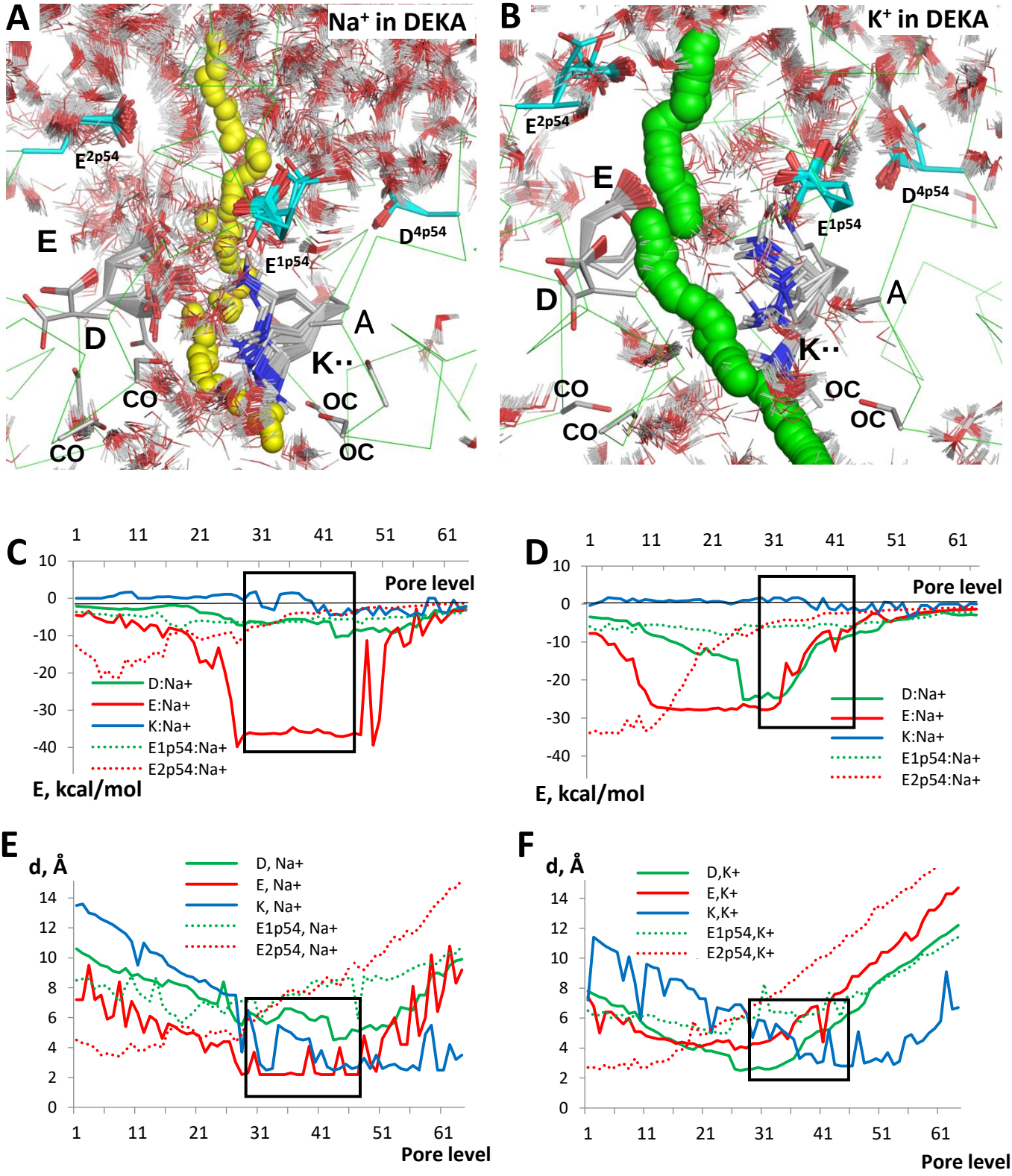


Figure S4. Na^+ and K^+ trajectories in Nav1.4 computed with Lys··, explicit solvent and the step of 0.25 Å. The DEKA region is boxed. Dielectric function $\varepsilon=d_{ij}$, was used (d_{ij} is distance between atoms). For clarity, radii of ions are reduced and carbon atoms of outer carboxylates are cyan

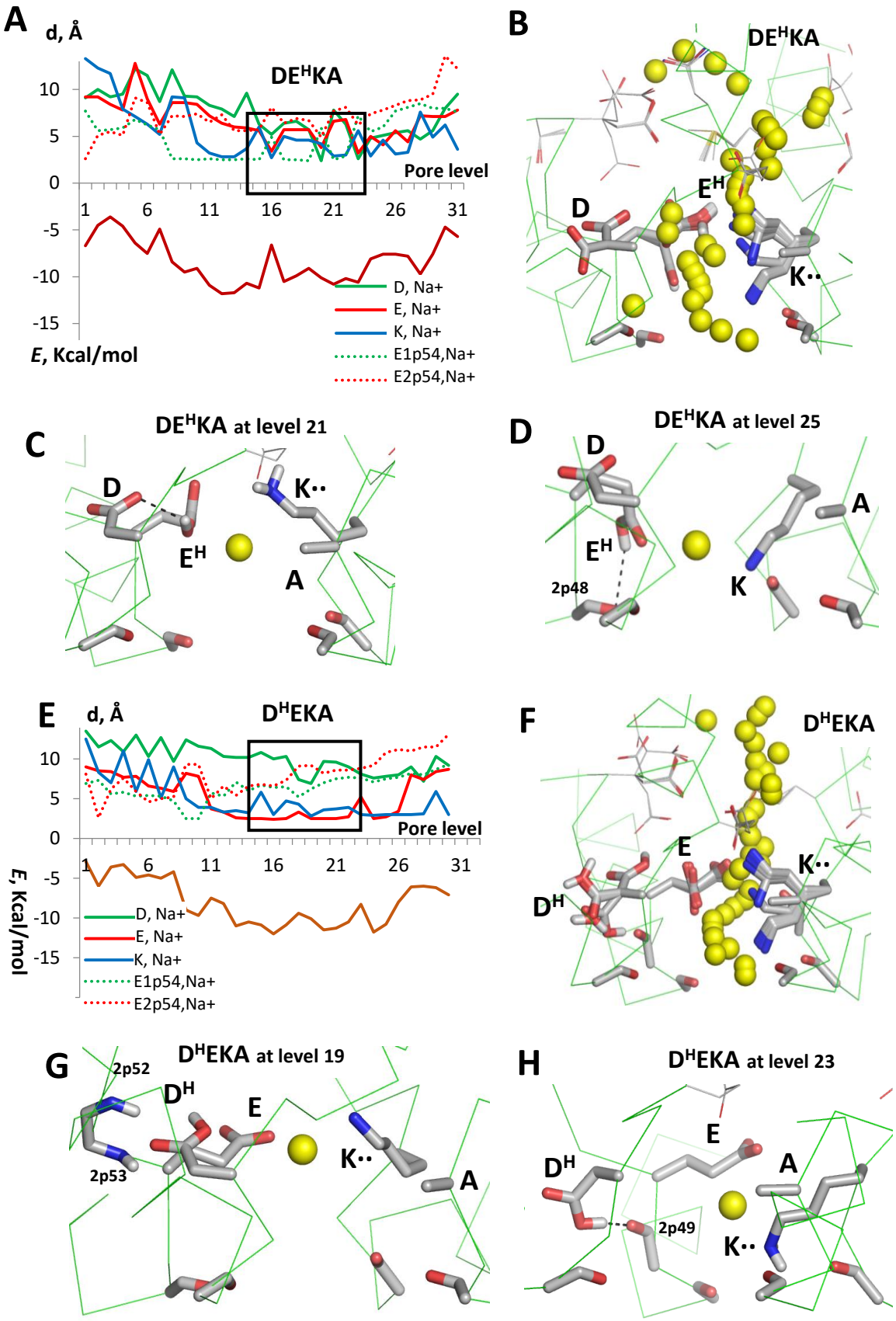


Figure S5. **Na⁺ trajectories in models with Glu^H (A-D) and Asp^H (*E-H*). *A,*** Na⁺ is chelated by Lys·· and E^{1p54} at levels 10-12 and 20, and by Lys·· and Glu^H at level 15. ***B,*** Superposition of AGMs at all steps of the Na⁺ trajectory. ***C***, At level 22 Glu^H donates an H-bond to Asp. ***D*** At level 25 Glu^H donates an H-bond to $^{O}C^{2p49}$ and Lys·· is dunked. ***E***, Lys·· and Glu⁻ chelate Na⁺ at levels 16 and 24-26. ***F,*** Superposition of AGMs at all steps of the Na⁺ trajectory. ***G,*** At level 19, Asp^H accepts H-bonds from backbone amides 2p52 and 2p53. ***H,*** At level 23, Lys·· is dunked, while Asp^H donates an H-bond to G^{2p49} . Similar H-bonds are implied in cryo-EM structures of hNav1.7 (Table 2)

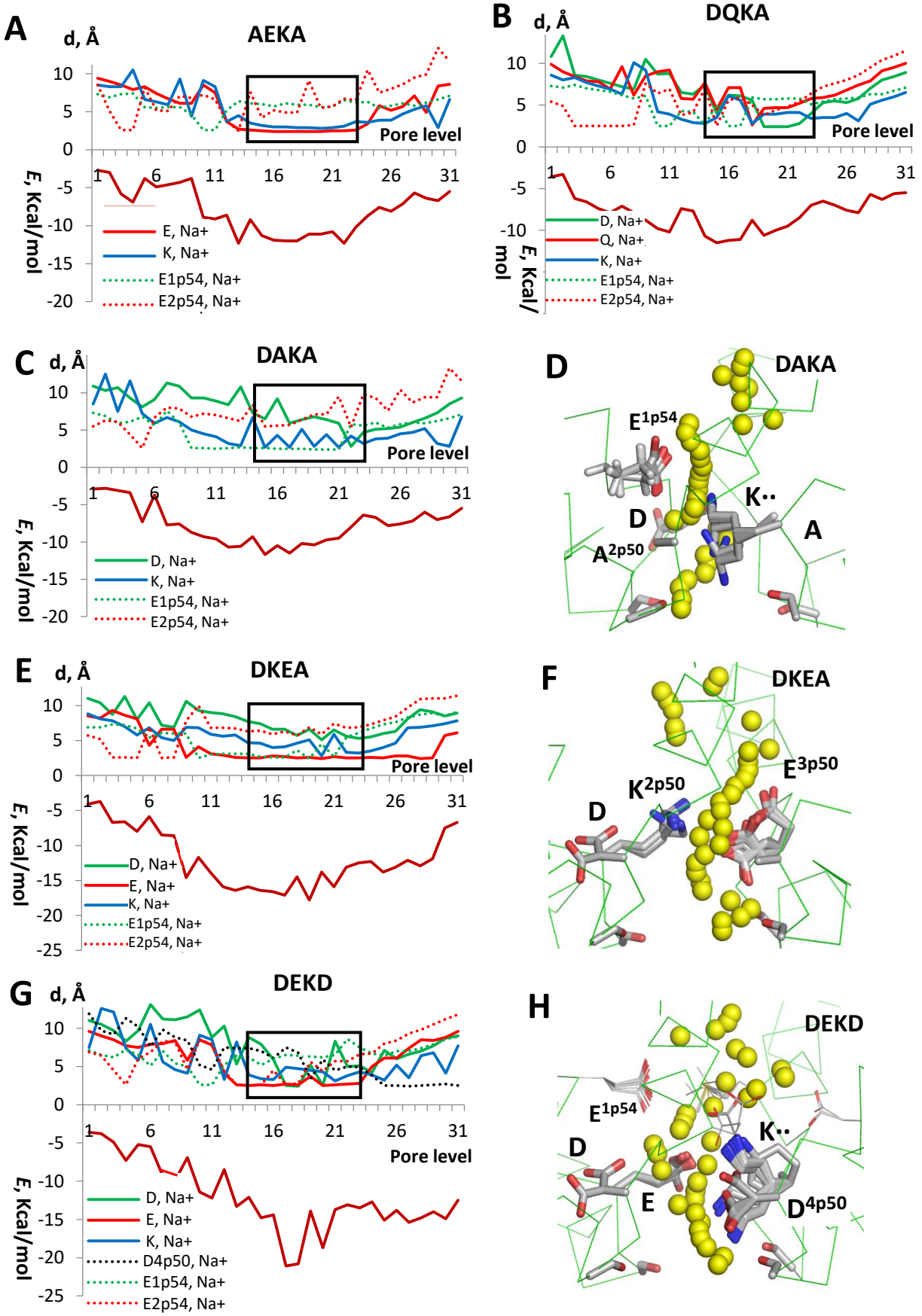


Figure S6. **Na⁺ trajectories in DEKA mutants with high or moderate Na/K selectivity. *A* (AEKA).** Lys·· and Glu chelate Na^+ at levels 14-21 and Lys·· dunks at level 30. ***B (DQKA).*** Lys·· chelates Na^+ with Q^{2p50} at level 18, with E^{1p54} or E^{2p54} at levels 4 and 15, and dunks at level 27. ***C, D* (DAKA).** Lys·· chelates Na^+ with E^{1p54} at levels 12, 14, 16, 18 and 20 and dunks at levels 29 and 30. ***E, F* (DKEA).** Lys·· in position *2p50* Glu^- in position *3p50* chelate Na^+ at levels 20 and 22-24, but Lys·· in position *2p50* does not dunk. ***G, H* (DEKD).** Na^+ is chelated by Lys·· and Glu^- at levels 13-15, 19 and by Lys·· and $^-D^{4p50}$ at level 24. Lys·· dunks at level 30. (See Table 4 for selectivity of the mutants).

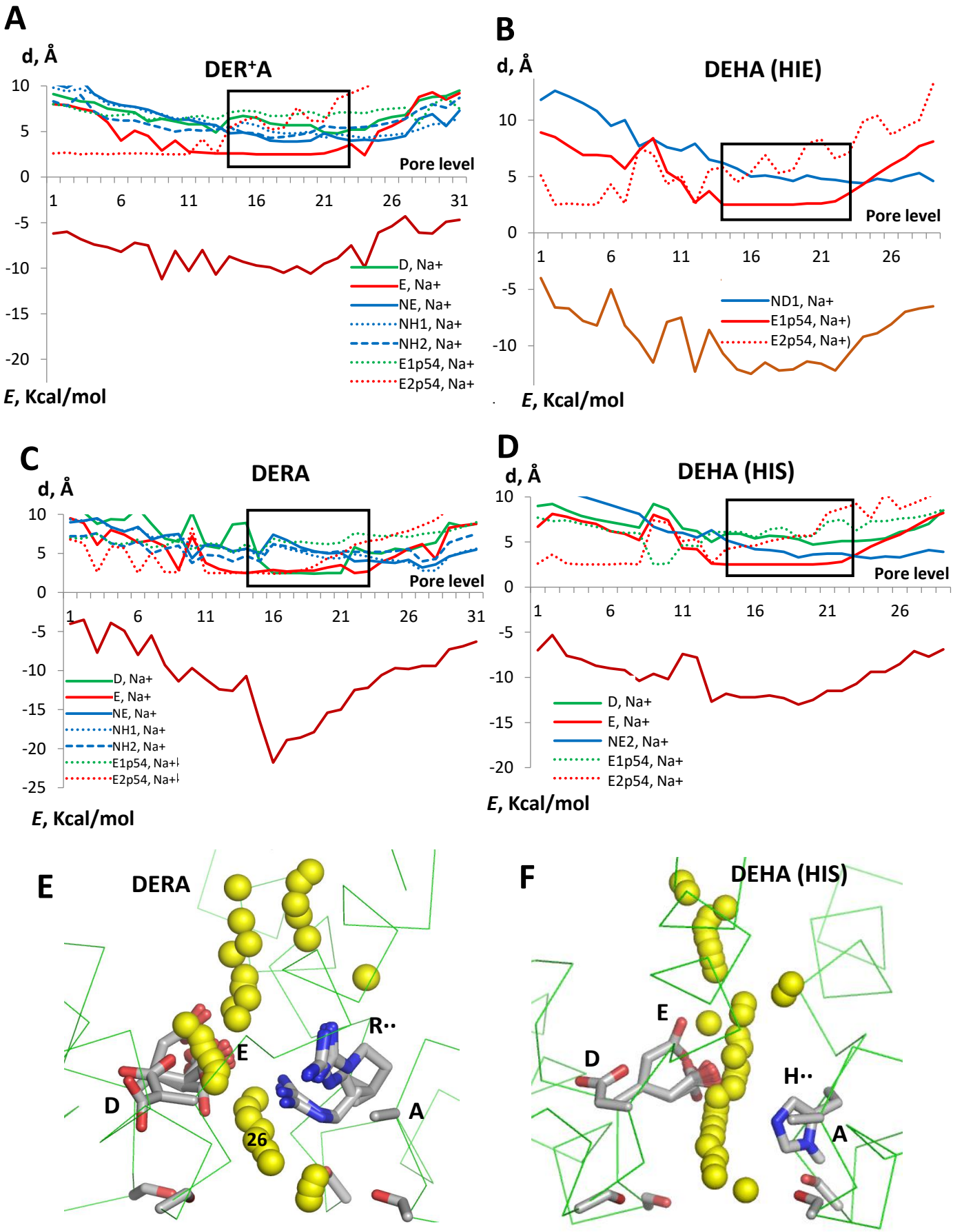


Figure S7. Na⁺ trajectories in the DARA and DAHA models. In models with protonated (***A***) and deprotonated (***C,E***) arginine, Na^+ does not contact any arginine nitrogen all along the ion trajectory. This can explain the lack of Na^+ selectivity in the DERA mutant (Table 4). ***B, D, F.*** In the models of two tautomeric forms of neutral histidine, Na^+ does not contact the histidine nitrogen and the DEKA carboxylate simultaneously. This can explain the lack of Na^+ selectivity in the DEHA mutant (Table 4).