Expanded View Figures

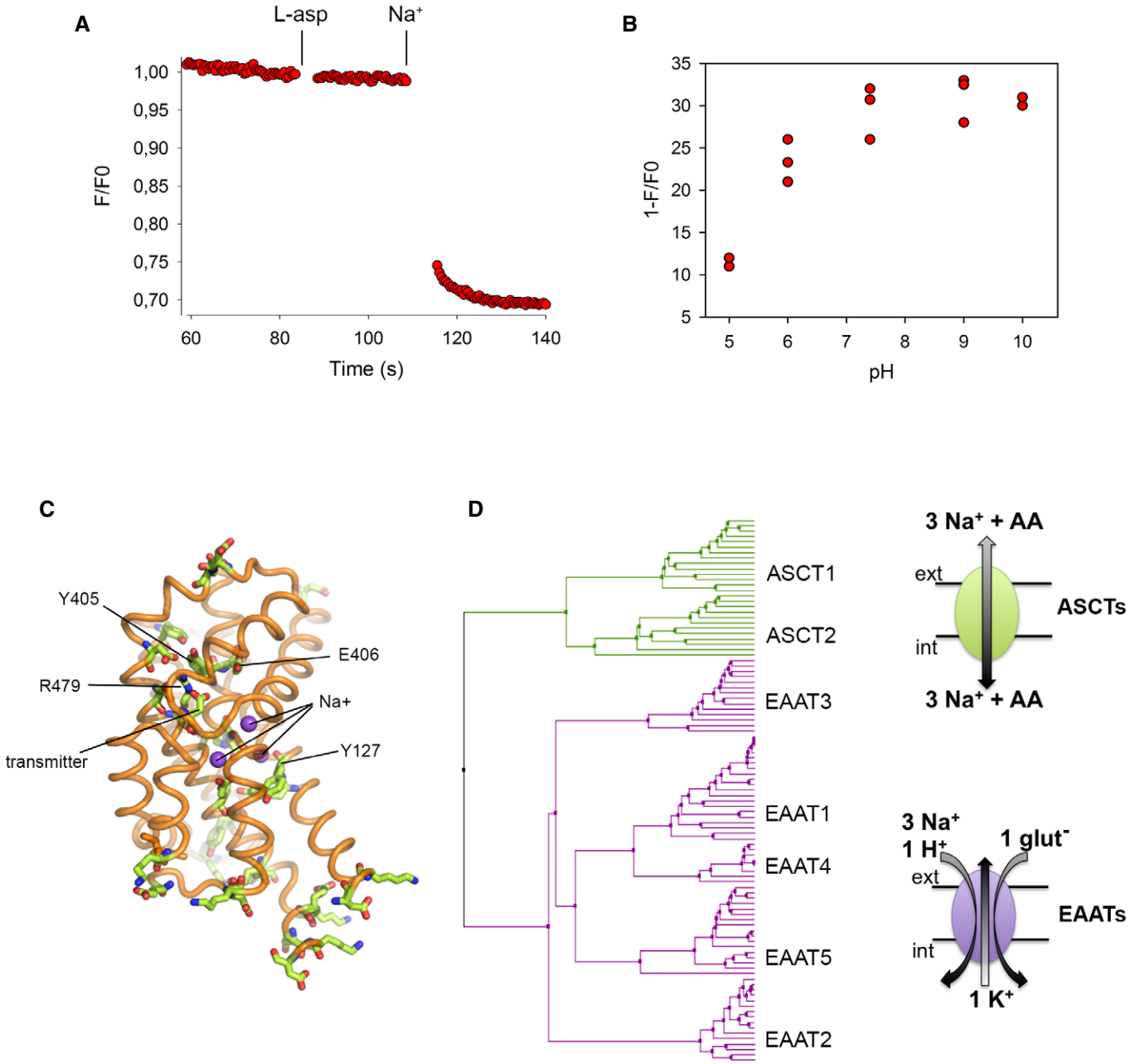

Figure EV1. Proton binding to conserved ionizable sidechain in EAAT1$_{CRYST}$.

A EAAT1$_{CRYST}$ tryptophan-fluorescence time course at pH 10 upon addition of saturating concentrations of L-asp and Na^+.

B Percentage of L-asp/Na^+-induced total fluorescence change ($1-F/F_0$) decreases at pH values below neutral.

C Out of > 30 ionizable sidechains (green sticks) in the tranD of EAAT1$_{CRYST}$ only four (labelled with residue number) are strictly conserved in EAATs, and not in ASCTs.

D Phylogenetic tree from 101 tranD sequences of representative vertebrate species (Gesemann *et al*, 2010) calculated with Jalview (BLOSUM62 average distance). Transport stoichiometry of EAATs and ASCTs vertebrate proteins is depicted in cartoon representation.

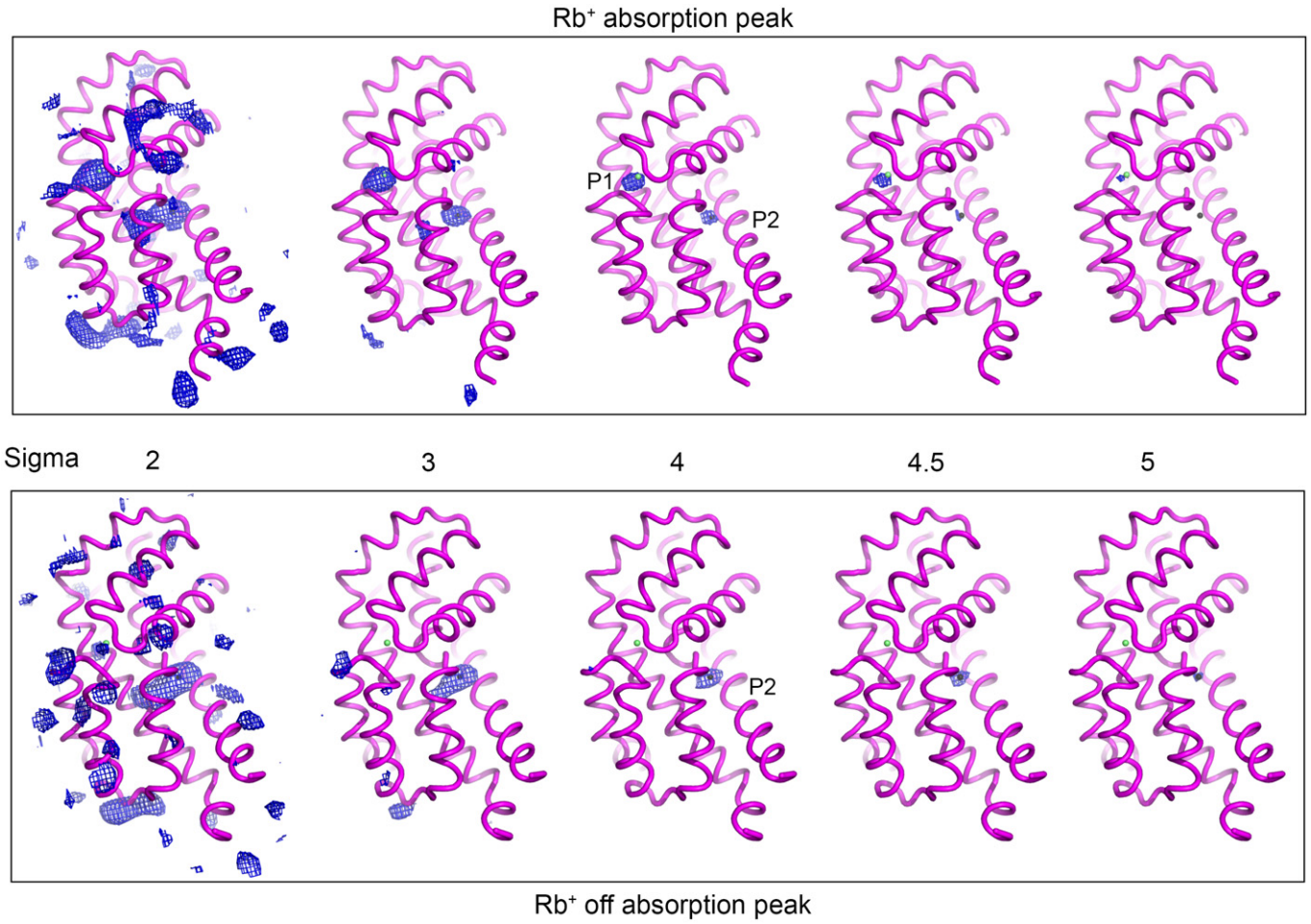

Figure EV2. Anomalous difference maps of Rb^+/Ba^{2+} bound structure.

Comparison of anomalous difference maps of Rb^+/Ba^{2+} bound $EAAT_{CRYST}$ structure at the Rb^+ absorption maximum (0.815 Å) and that of $EAAT_{CRYST-II}$ off the Rb^+ absorption maximum (0.998 Å). Maps are contoured at increasing sigma levels around the tranD. Rb^+ (green sphere) and Ba^{2+} (black sphere) bound to the transporters are depicted.

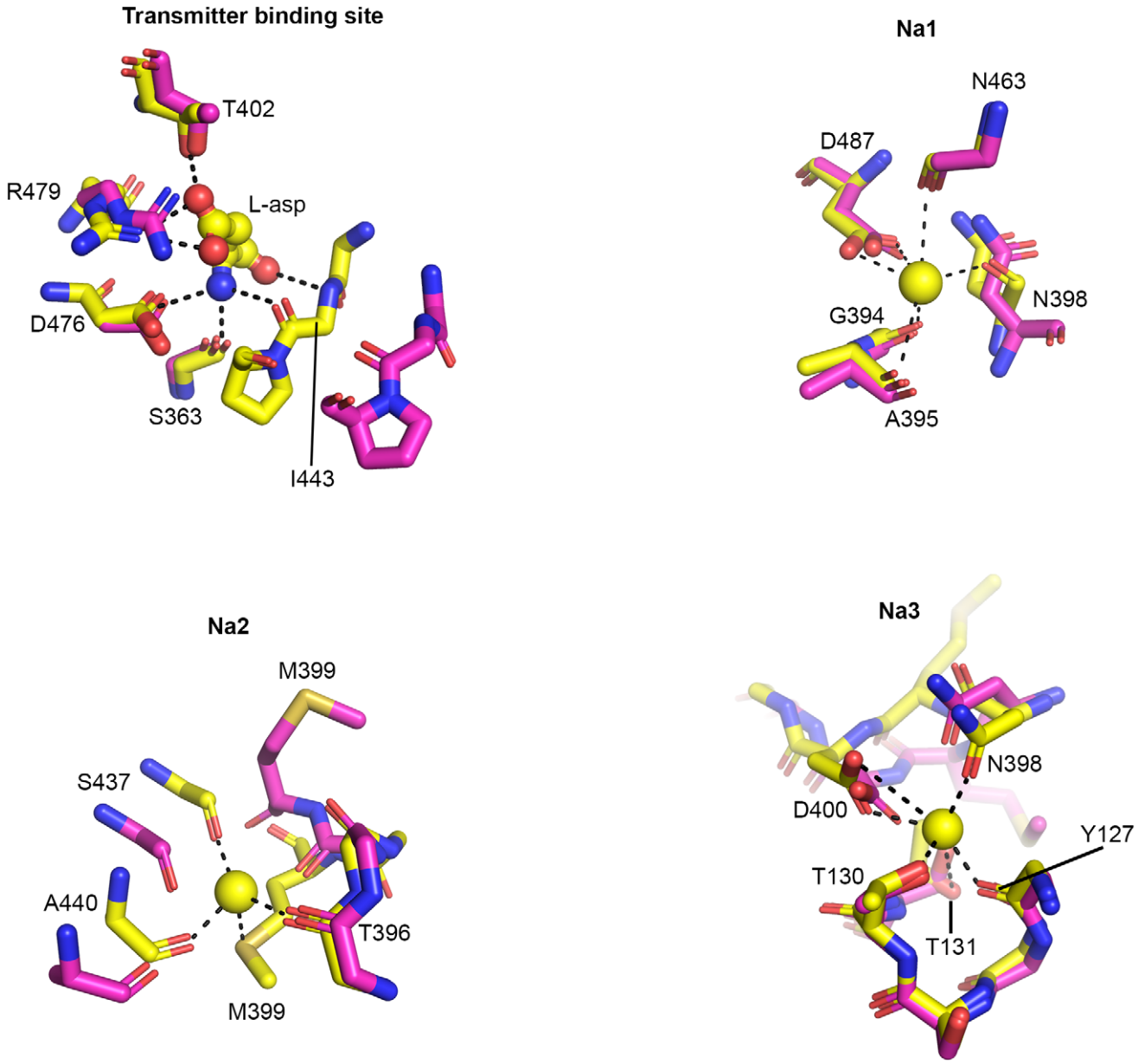

Figure EV3. Transmitter and sodium binding sites in Na^+/transmitter- and Rb^+ bound structures.

Conformational re-arrangements around the transmitter and three Na^+ binding sites in Na^+/transmitter- (yellow) and Rb^+ bound (pink) $EAAT_{CRYST}$ structures.

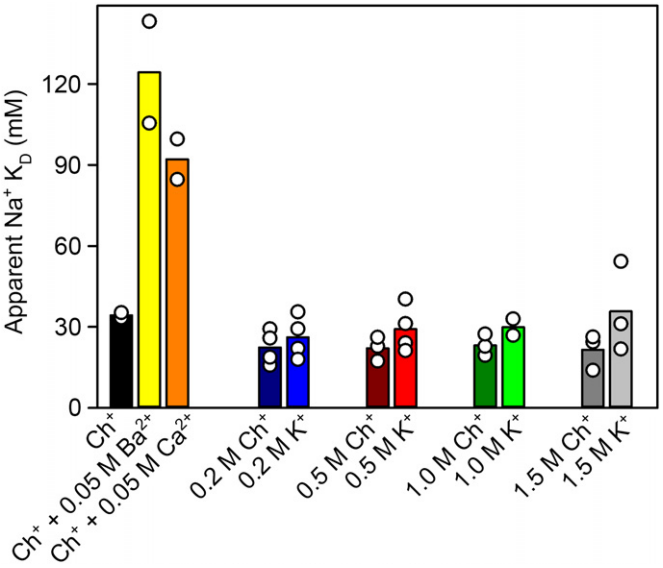

Figure EV4. Na^+ titrations in different ionic conditions.

$EAAT1_{CRYST}$ apparent Na^+ K_D significantly increased upon addition of 50 mM Ba^{2+} or Ca^{2+} to a choline (Ch^+)-based cuvette buffer, but were not modified when Ch^+ in the buffer was substituted for K^+ at concentrations up to 1.5 M. Bar plots present average of at least two independent experiments (empty circles).

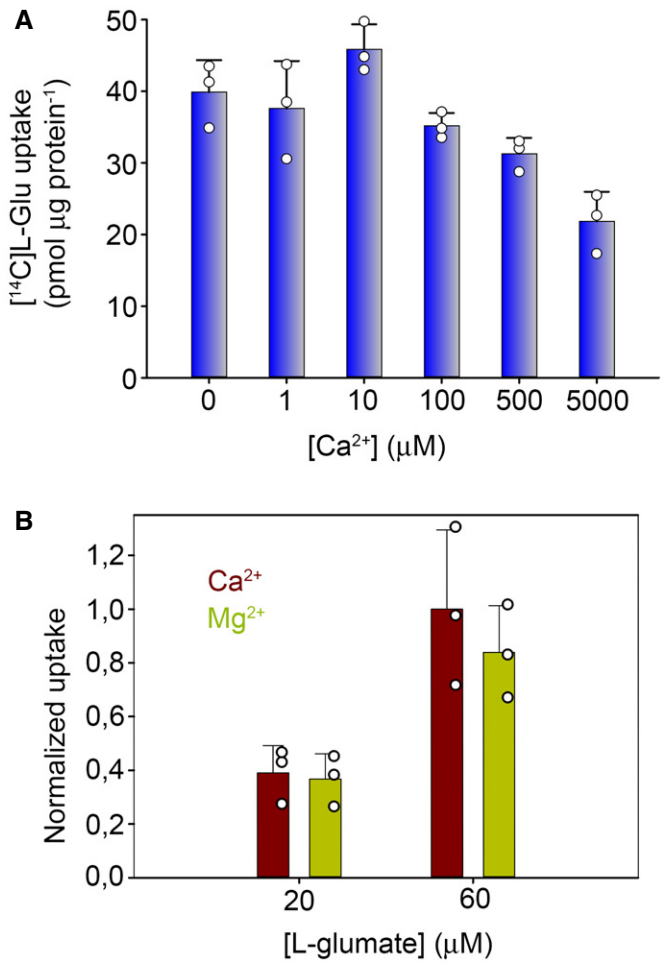

Figure EV5. Ca^{2+} effect on steady-state transmitter transport.

A Effect of intra-liposomal Ca^{2+} on L-glutamate transport in the presence of opposite gradients of Na^+ and K^+.

B Effect of 5 mM extracellular Ca^{2+} on L-glutamate transport in cells expressing $EAAT1_{CRYST}$. There was not significant change in Na^+-induced L-glutamate uptake upon substitution of extracellular Ca^{2+} for Mg^{2+}, a divalent cation that does not induce changes in Trp fluorescence or compete for Na^+ (not shown).