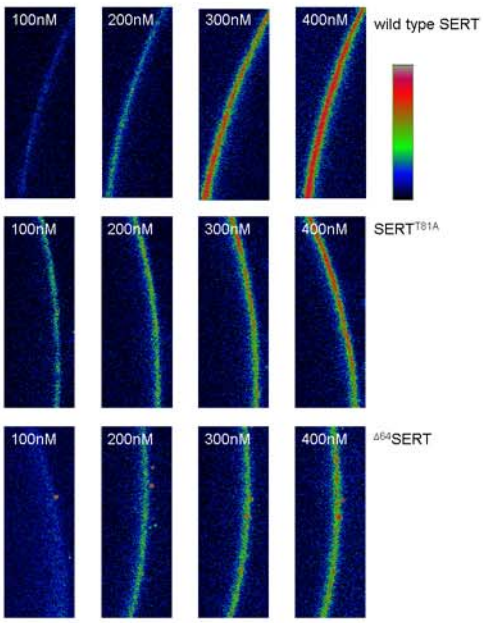
Supplementary Figures:

Supplementary Figure A: Expression level of wild type SERT and mutants in Xenopus laevis oocytes using the Rhodamine-labeled benztropine JHC1-64.
Xenopus laevis oocytes expressing wild type SERT, $SERT^{T81A}$ and $^{\Delta 64}$SERT were studied using confocal microscopy as described under 'Experimental Procedures'. Concentration of the Rhodamine-labeled benztropine JHC1-64 is indicated in each picture. All oocytes were checked for expression by measuring PCA-induced currents prior to the experiment under two-electrode voltage clamp conditions (*data not shown*).

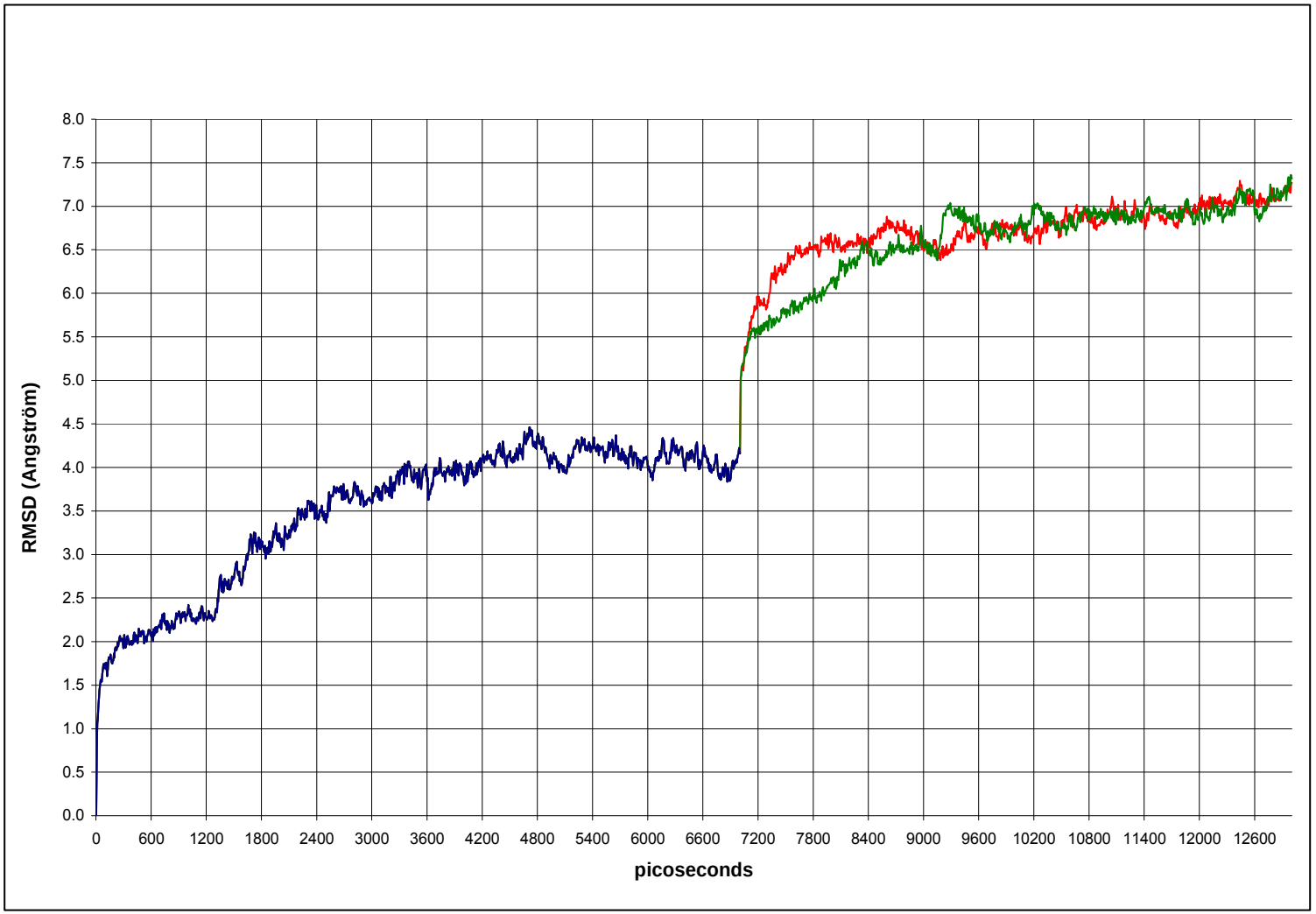
Supplementary Figure B: Course of the root mean square deviations (RMSD) of the protein backbones during MD simulations. RMSD of the wild type SERT (displayed in blue) and after mutation of to $SERT^{T81A}$ (displayed in red) or $SERT^{T81D}$ (displayed in green).

Supplementary Figure C: Amphetamine-induced [^{3}H]5HT release by the $^{\Delta 64}$SERT truncation mutant expressed in *Xenopus laevis* oocytes.
PCA-induced release of [^{3}H]5HT in cRNA-injected *X. laevis* oocytes under current clamp conditions (n=2, from two individual experiments). The dashed line is a representative wild type SERT trace, adapted from Fig. 5. The inset graph illustrates the membrane potential recorded during release experiments under current clamp conditions, as described under 'Experimental Procedures'.

Supplementary Figure A



Supplementary Fig. B



Supplementary Fig. C