Time resolved UV/Vis spectroscopy on xenorhodopsin variants

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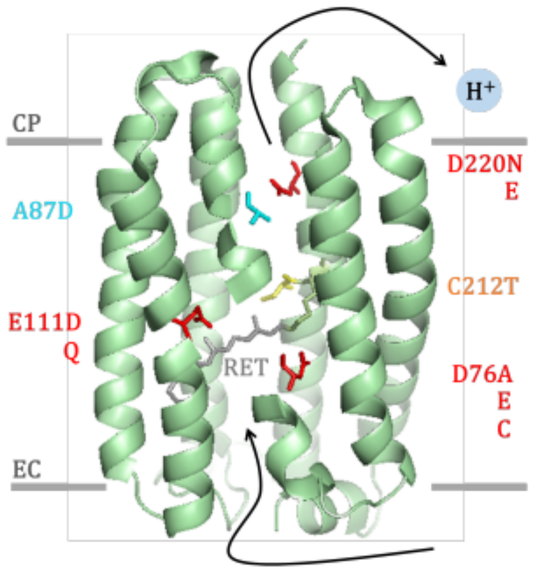
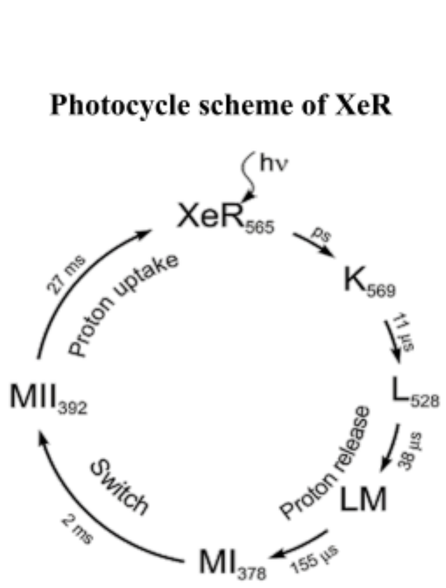
Introduction



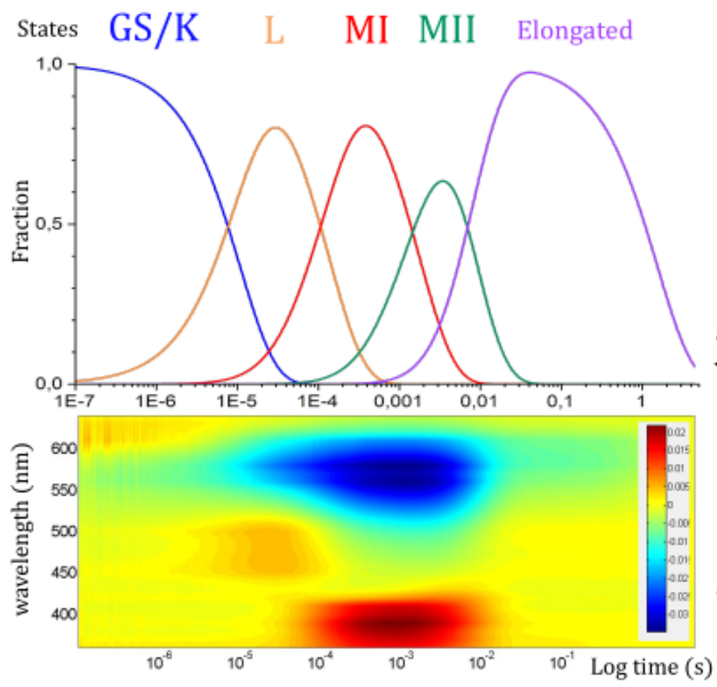
Structure of xenorhodopsin (XeR). Based on preliminary spectroscopic data and alignment to bacteriorhodopsin, we generated point mutations on higlighted residues. (PDB: 6eyu)

Photocycle scheme of XeR



Reference Vitaly Shevchenko et. al. 2017. Inward H+ pump xenorhodopsin: Mechanism and alternative optogenetic approach. *Science Advances.*

Wild type

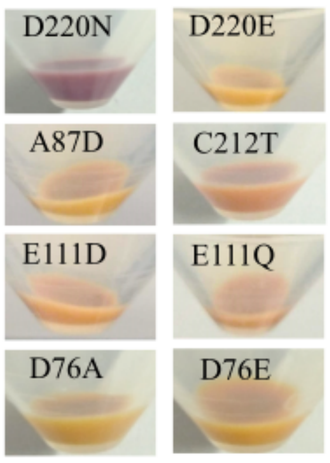


	τ
K → L	10.5 µs
L → MI	131 µs
MI → MII	1.71 ms
MII → EL	7.08 ms
EL → GS	1.49 s

▲ ◀ **Fitted intermediates states in XeR photocycle.** The plots show arise of different intermediate states. According to our results, we propose an extra elongated state. The table shws relative time constant of the kinetics.

◀ **Transient absorption changes across wavelengths and time.**

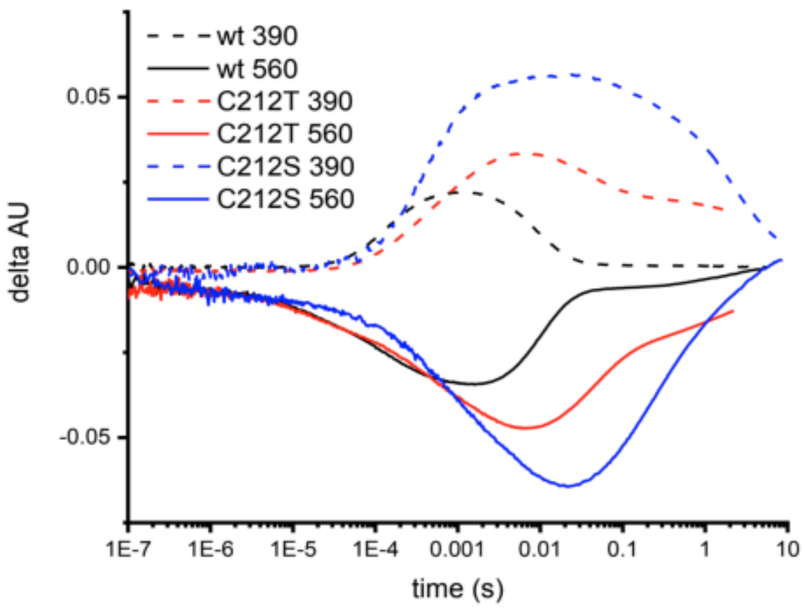
Cysteine near retinal Schiff base

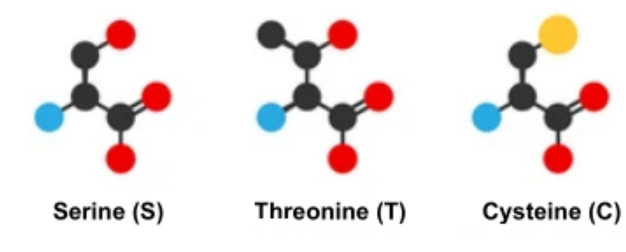


E. coli **cells with *Ns*XeR variants expressed.**

Variants	λmax (nm)
WT	565
C212T	551
C212S	554

Max absorption of C212 variants. Both mutant on C212 result in a larger than 10 nm blue shift of retinal absorption.



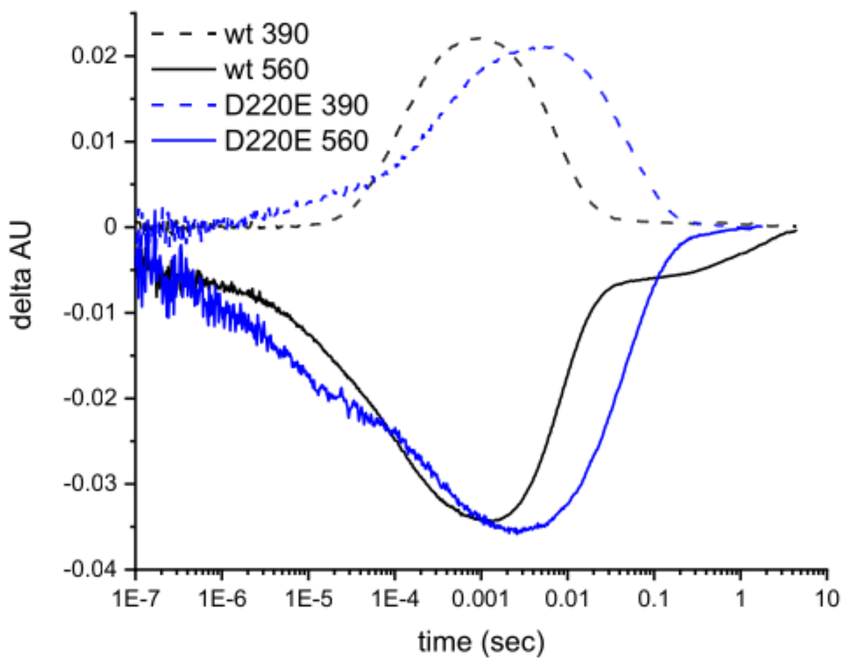


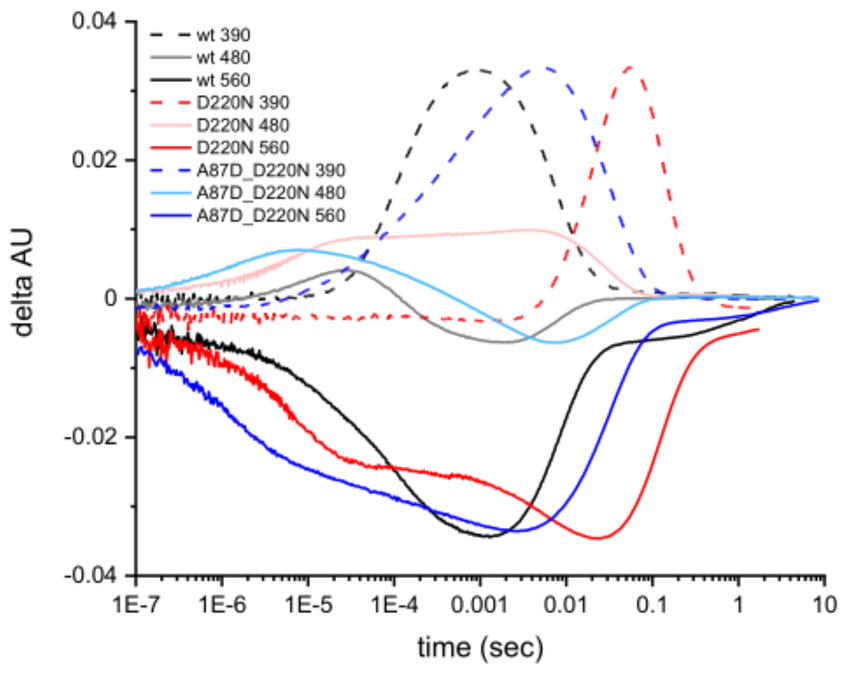
Time resolved visible spectrscopy on C212 residue. The kinetics of XeR wild-type (**black**), mutant C212T (**red**) and C212S (**blue**) on absorption of 560 nm (ground state) and 390 nm (M state). Both mutants show slowed M states formation, which may imply that the proton uptake in the late stage is interupted. On the right side, it shows the molecular strucutre of amino acids which we exchanged to.

Proton Receptor Exchange

Mutants	λmax (nm)
WT	565
A87D	553
A87D_D220N	561
D220N	562
D220E	565

Max absorption of A87 and D220 variants. The mutagenesis on D220, which is quite far away from retinal, doesn‘t change too much of the retinal absorption. However, the mutation on A87 is blue shifted, which is not observed in the double mutation of A87D_D220N.





Time resolved visible spectrscopy on C212 residue. The kinetics of XeR wild-type is compared to variant D220E, D220N and A87D_D220N. The absorption of 560 nm (ground state) and 390 nm (M state) were monitored. The measurement was done in environment of 100 mM NaCl, 50 mM sodium phosphate, 0.1% DDM, pH 7.4. The elongated state of D220E variant is diminished. The largely delayed M state in D220N was rescued by putting a proton receptor in close site (A87).