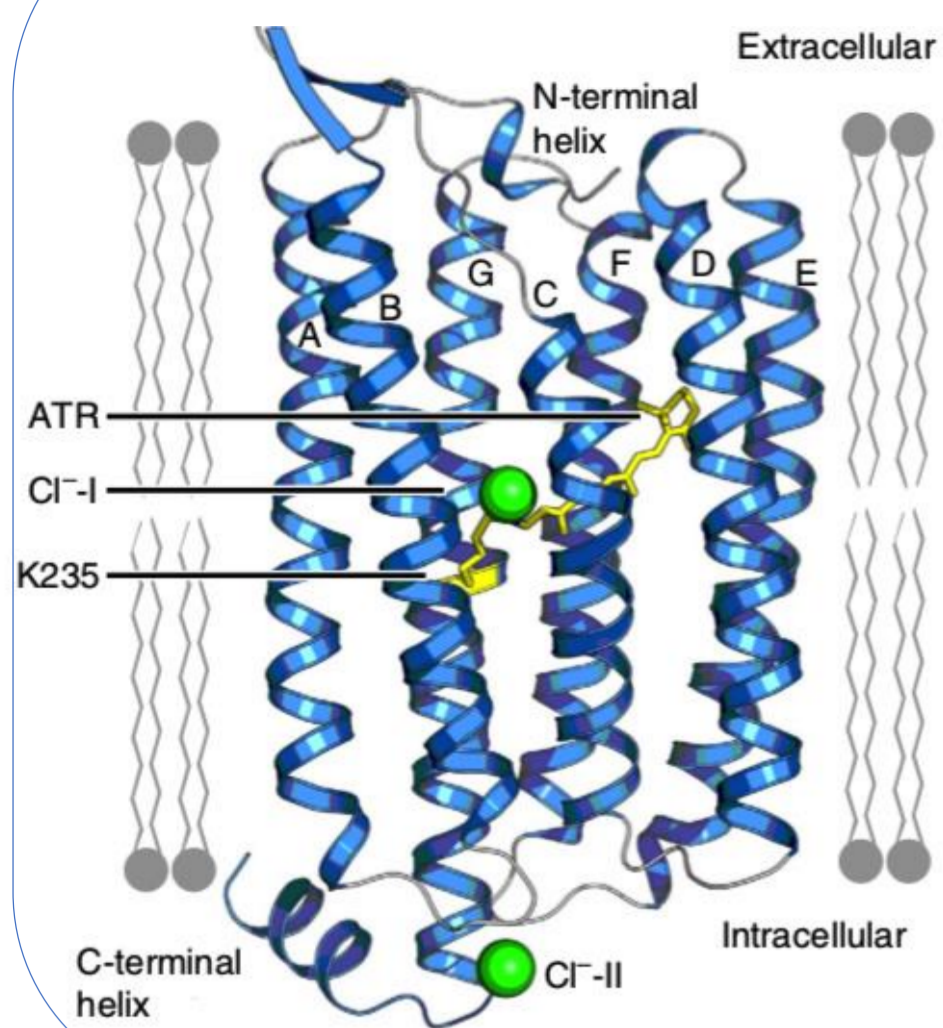


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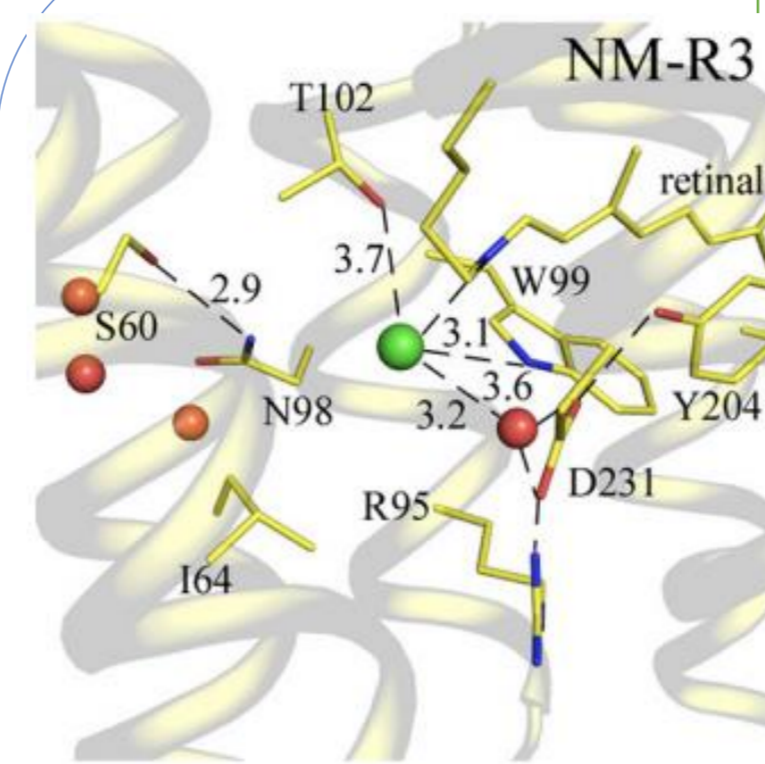
INTRODUCTION



The light-activated *NmHR* rhodopsin pumps Cl⁻ ions into the cell, representing the first chloride-pumping rhodopsin uncovered in a marine bacterium.

Two crystal structures revealed the presence of two chloride ions, Cl-1 lies close to the Schiff base and Cl-2 in an extracellular surface pocket that may be involved in a potential Cl⁻ vectorial transport.

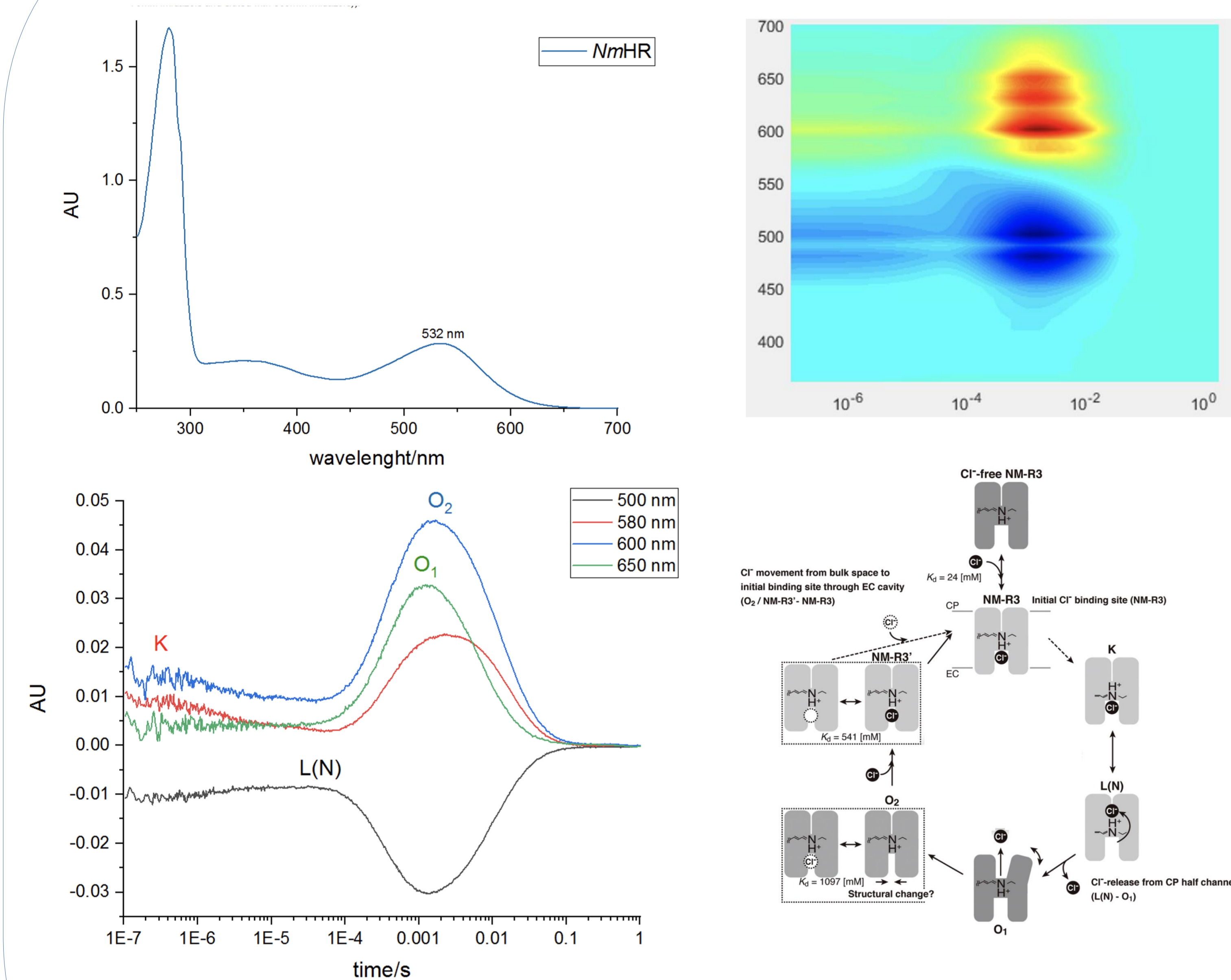
SCOPE OF THIS WORK AND EXPERIMENTAL DESIGN



No. in NM-R3	95	98	102	109	197	213	223	231	235		Max absorption (nm)	
No. in NpHR	123	126	130	137	218	234	244	252	256		<i>NmHR</i> wt	532 nm
No. in BR	82	85	89	96	178	194	204	212	216		<i>NmHR</i> D231E	550 nm
BR	(R)	D	T	D	T	E	E	(D)	K		<i>NmHR</i> D231C	564 nm
NpHR	(R)	T	S	A	T	E	T	(D)	K		<i>NmHR</i> D231N	554 nm ⊕
NM-R3	R	N	T	Q	M	F	R	D	K		<i>NmHR</i> R95A	540 nm ⊕
KR2	(R)	N	D	Q	F	T	R	(D)	K			

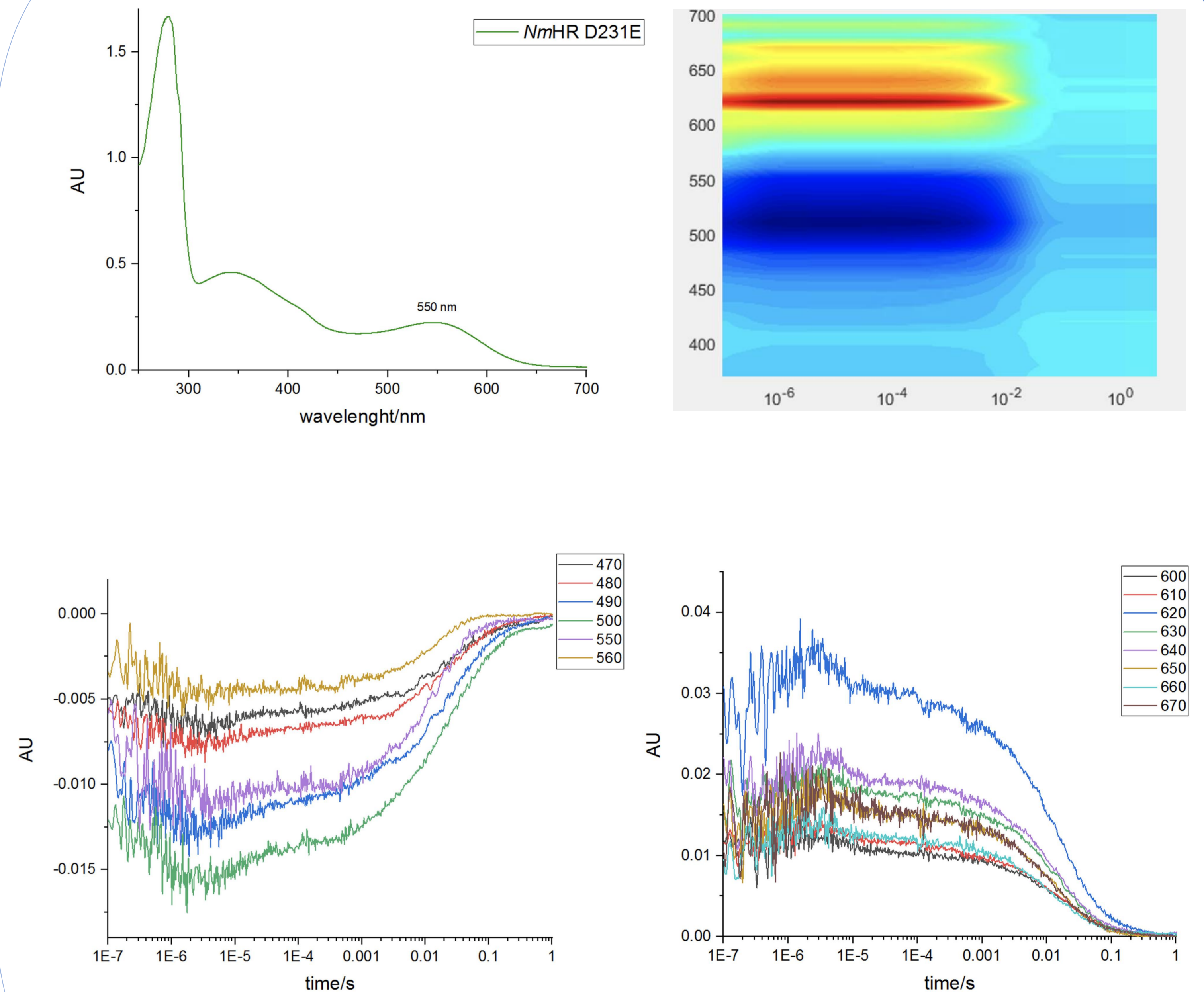
Study the function of the conserved charged aminoacids pair D231/R95 in the chloride transport pathway, using molecular biology and spectroscopic techniques such as mutagenesis, flash photolysis and FTIR.

FLASH PHOTOLYSIS *NmHR* WT

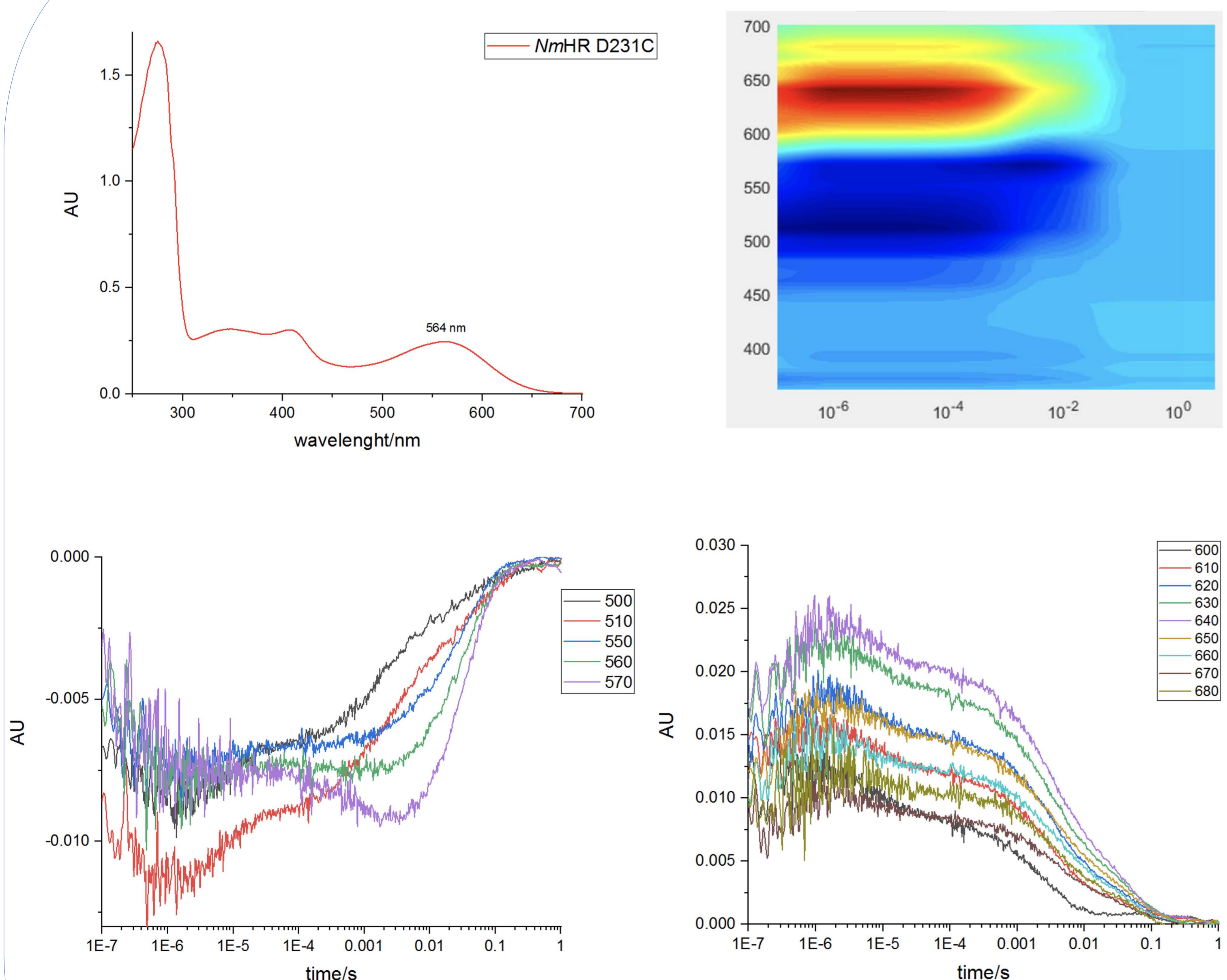


The release and uptake of Cl⁻ occur in the early and in the later stages of the photocycle, respectively.

NmHR D231E

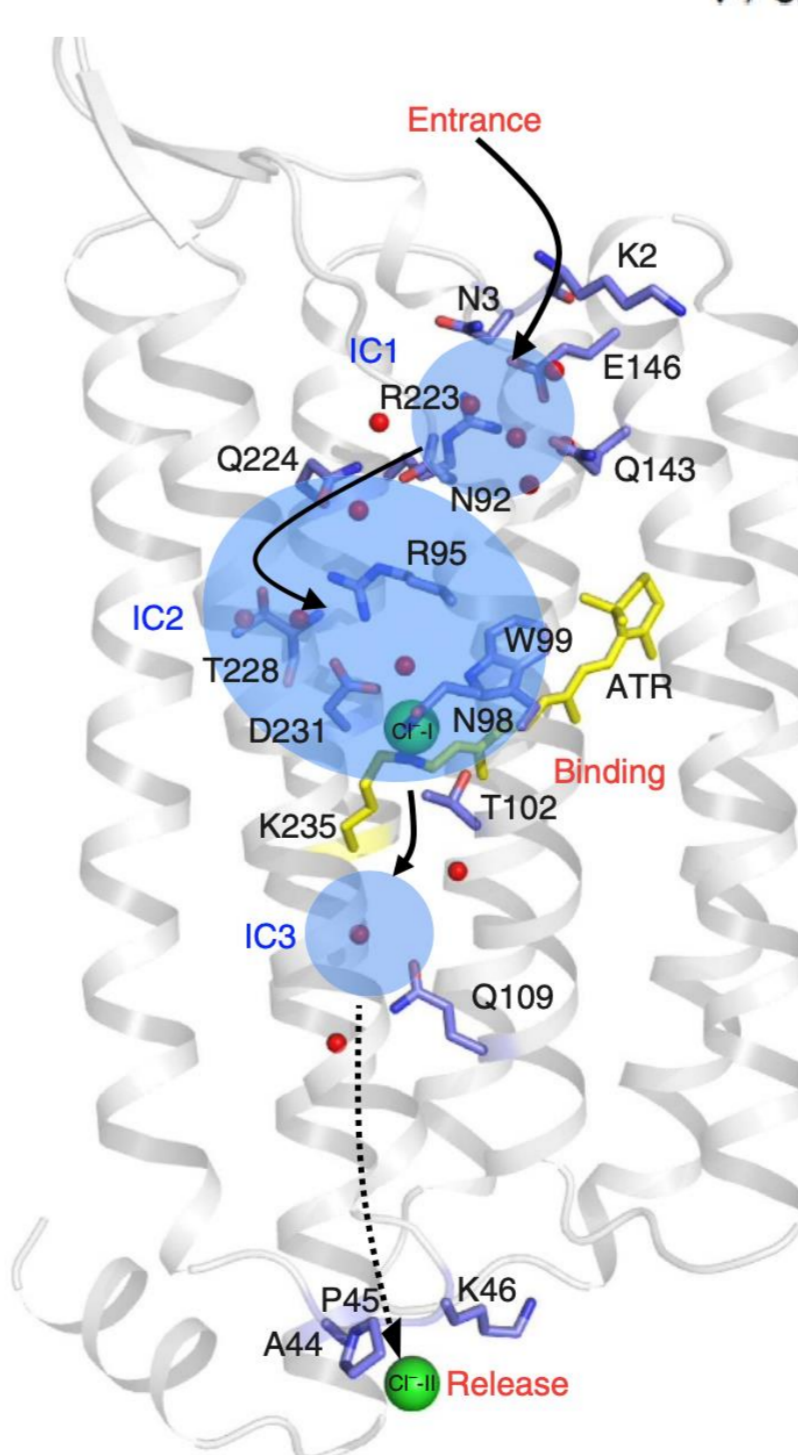
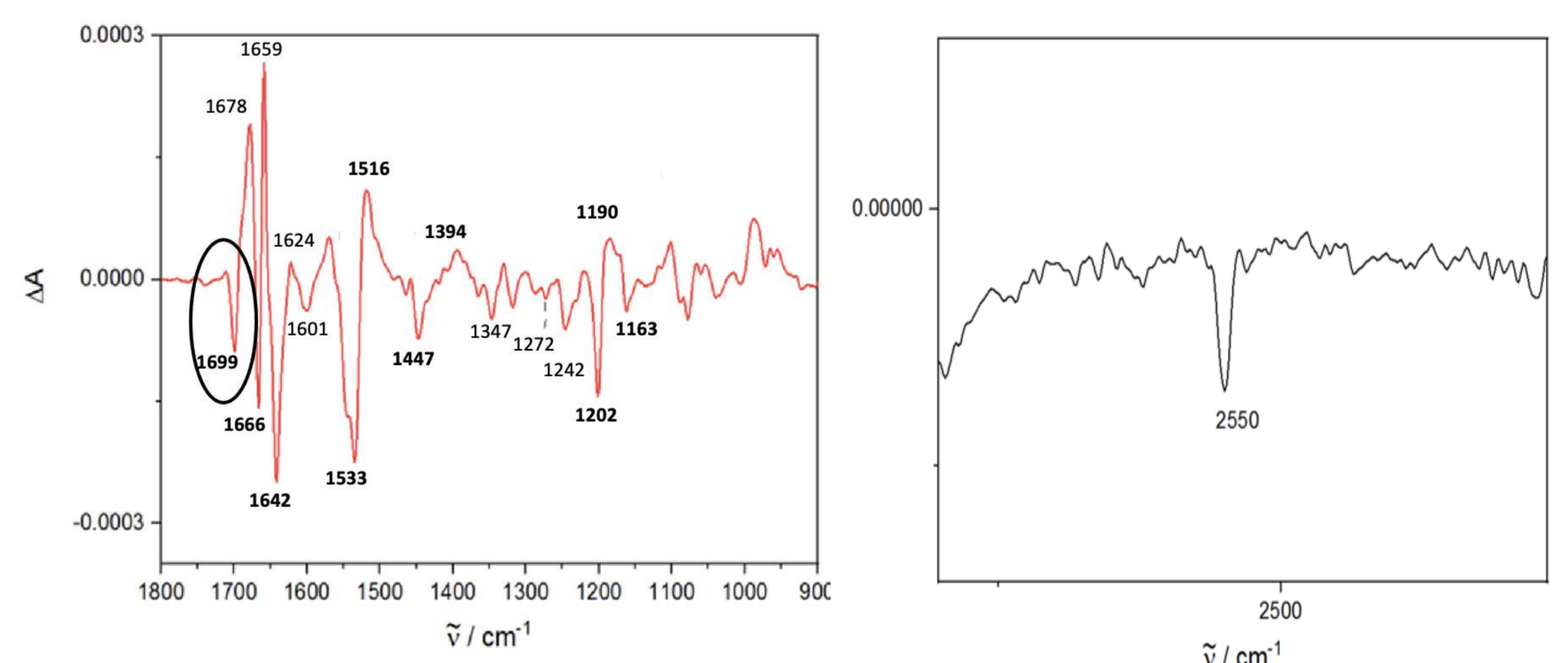


NmHR D231C



The time evolution of the flash-induced absorption changes of the D231E and D231C mutants show that the photocycle is strongly impaired and trapped in the early stages (the chloride ion cannot be released).

STEADY STATE FTIR *NmHR* WT



FUTURE PERSPECTIVES:

- FTIR on the C55-C105 and N92-N98 mutants
- Chemical rescue on D231C and R95A mutants
- Nanodiscs and lipid reconstitution

References:

Kuglae K, et al., Nature Comm., 2016, 24, 7:12677. Tsukamoto T, et al., J. Phys. Chem., 2017, 121, 2027-2038. Hosaka T, et al., Journal of Mol. Biol., 2016, 291, 17488-17495