

## Supplementary Materials for

### Structure and mechanisms of sodium-pumping KR2 rhodopsin

Kirill Kovalev, Vitaly Polovinkin, Ivan Gushchin, Alexey Alekseev, Vitaly Shevchenko, Valentin Borshchevskiy, Roman Astashkin, Taras Balandin, Dmitry Bratanov, Svetlana Vaganova, Alexander Popov, Vladimir Chupin, Georg Büldt, Ernst Bamberg\*, Valentin Gordeliy\*

\*Corresponding author. Email: valentin.gordeliy@ibs.fr (V.G.); ernst.bamberg@biophys.mpg.de (E.B.)

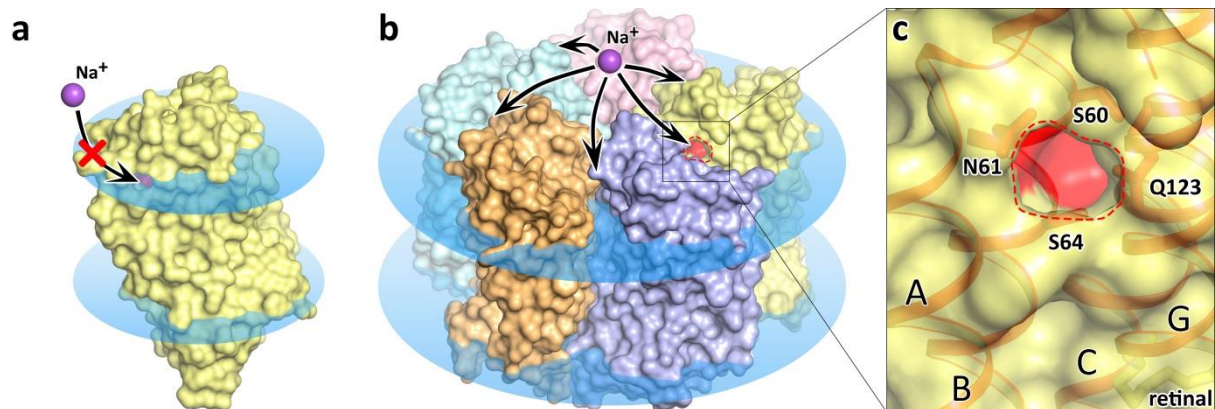
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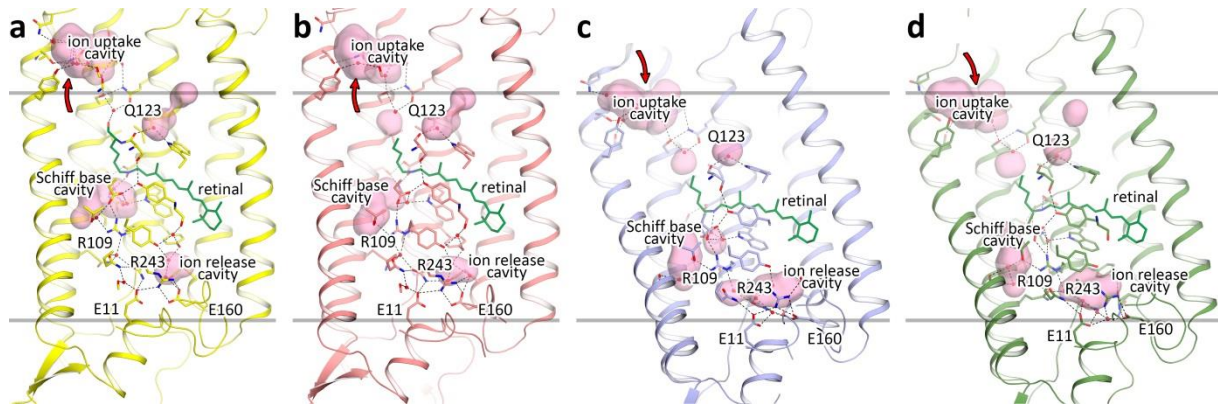
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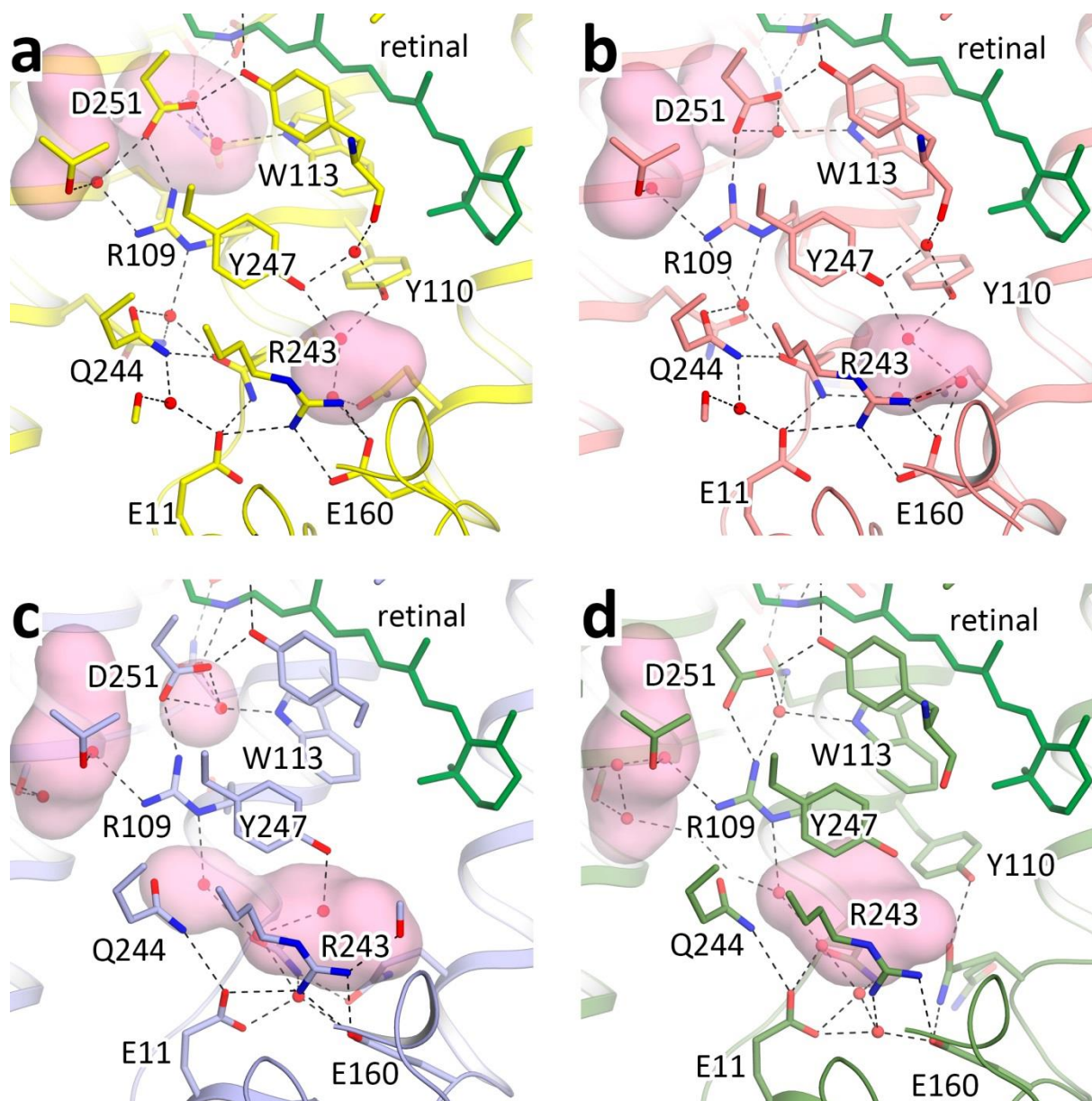
## Supplementary materials



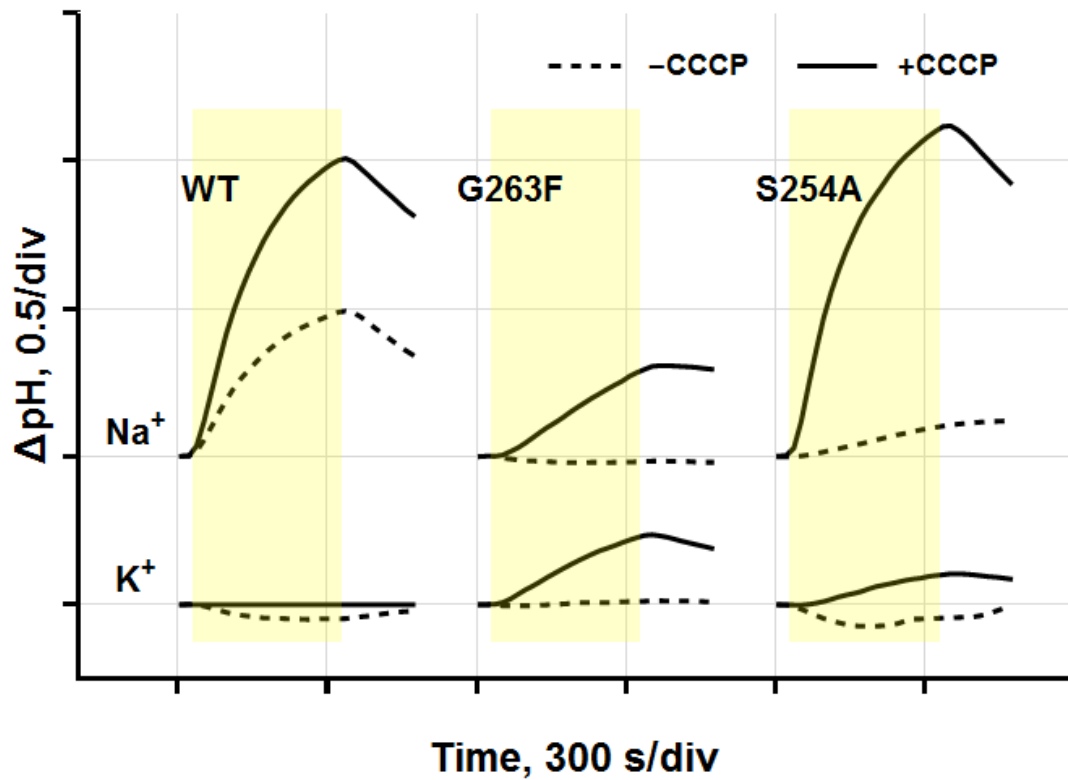
**Fig. S1. KR2 ion uptake pore.** **a.** Overall surface view of KR2 monomer in the membrane. Pore entrance is contoured red. Red contoured cross shows the blocking of the sodium passage due to the ion uptake vestibule burying into the membrane. **b.** Overall surface view of KR2 pentamer in the membrane. Pore entrance is contoured red. Ion uptake vestibule is positioned in the cytoplasmic space and is accessible for sodium. The hydrophobic membrane core boundaries are shown with the blue planes. **c.** Detailed view of the pore. Asn61, Gln123, Ser60 and Ser64 are colored red and located in the bottom of the cavity. The pore is formed by A and G helices.



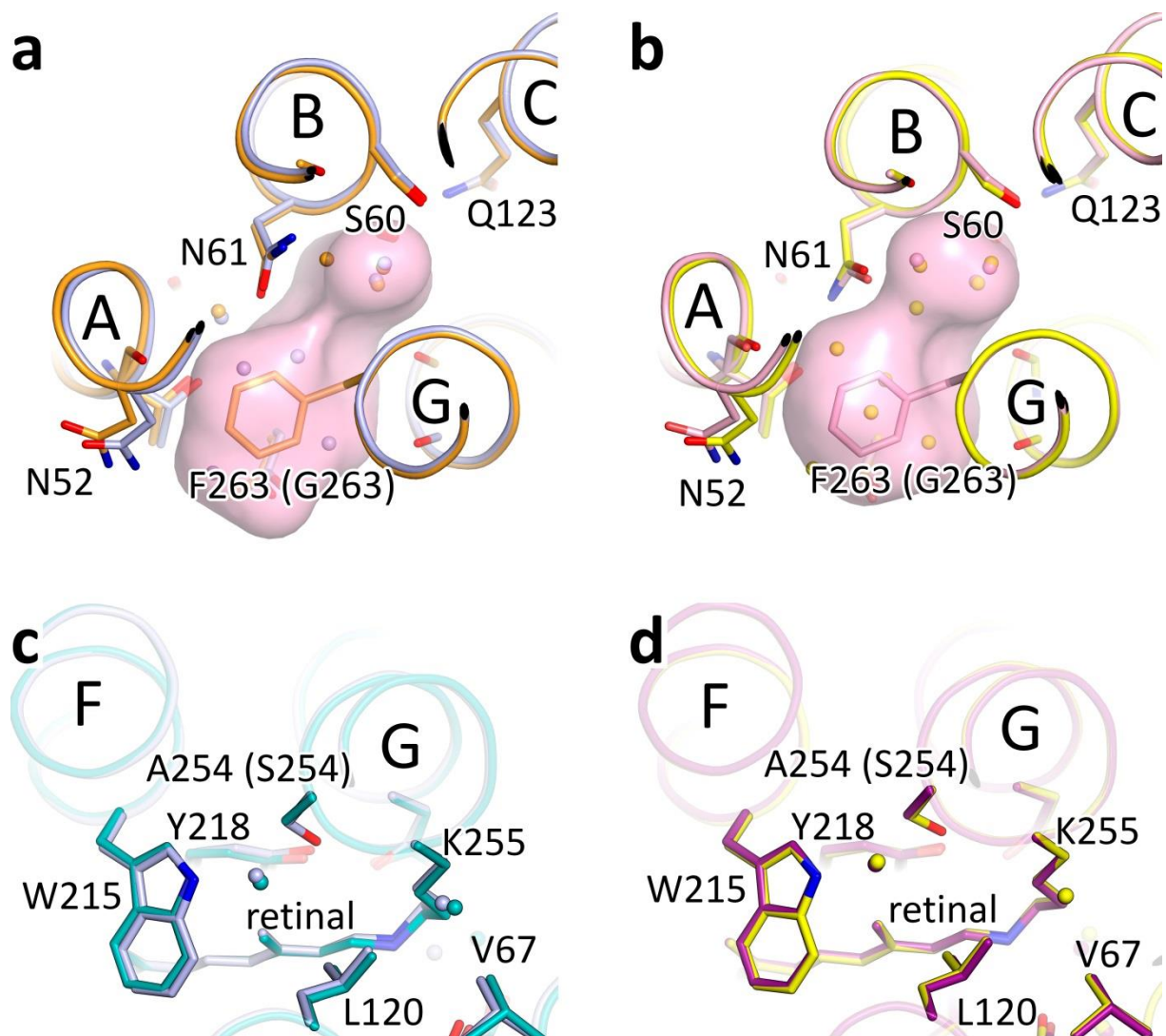
**Fig. S2. Comparison of orientations and cavities inside different KR2 structures. a.** Structure of chain A of pentameric  $\text{Na}^+$  pumping form (expanded conformation, pH 8.0, present work) is shown in yellow. **b.** chain E of 4XTN model (compact conformation, pH 4.9) is shown in salmon. **c.** 4XTL model (compact conformation, pH 4.3) is shown in light blue. **d.** 3X3C model (compact conformation, pH 7.5-8.5) is shown in green. Red contoured arrows show the important change in the position of the ion uptake cavity relative to the cytoplasmic side in case of pentameric form. The hydrophobic membrane core boundaries are shown with the black lines. The cavities are colored pink. A prosthetic group retinal is colored dark green.



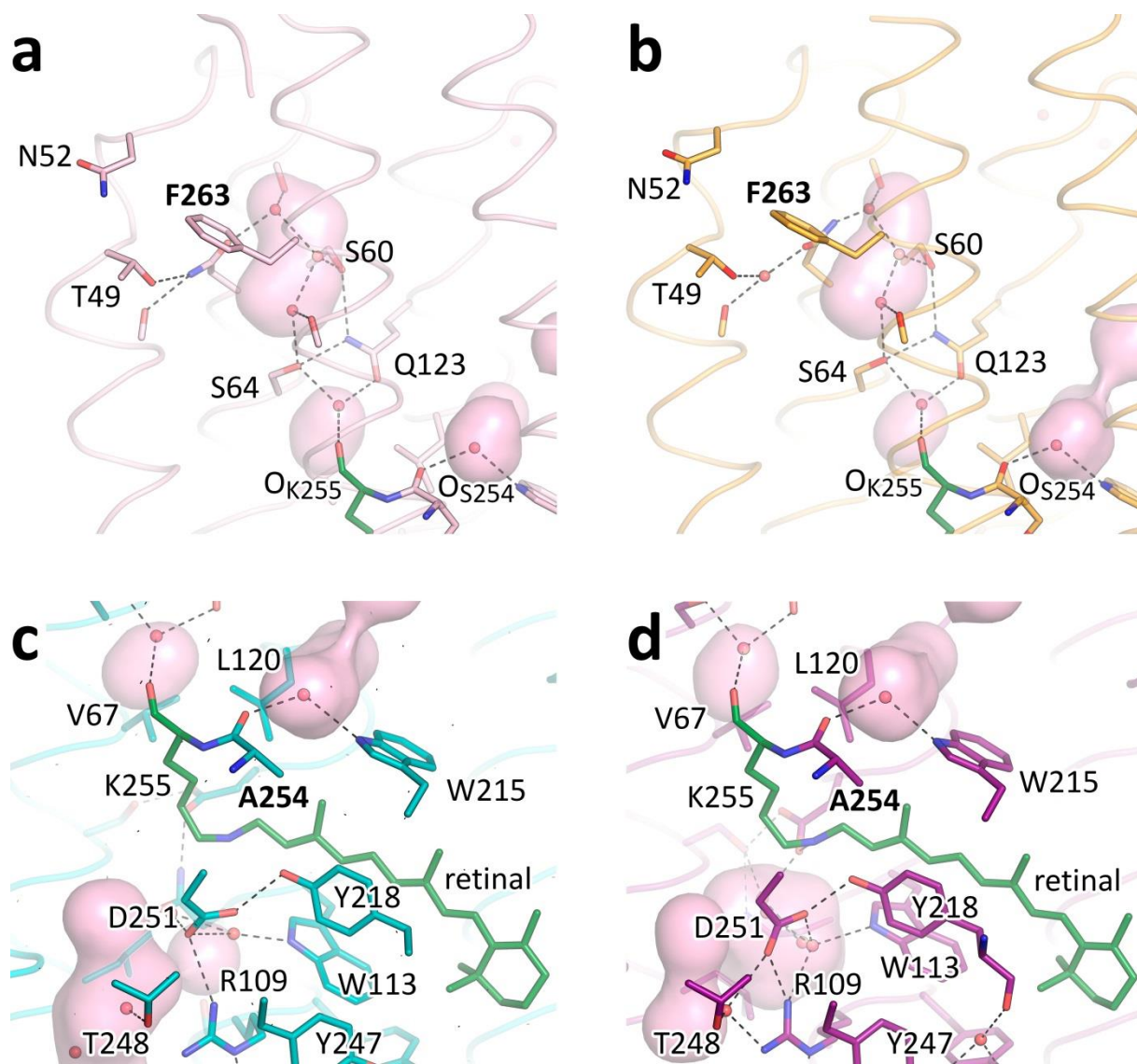
**Fig. S3. Comparison of extracellular regions of different KR2 structures.** **a.** Structure of chain A of pentameric  $\text{Na}^+$  pumping form (expanded conformation, pH 8.0, present work) is shown in yellow. **b.** chain E of 4XTN model (compact conformation, pH 4.9) is shown in salmon. **c.** 4XTL model (compact conformation, pH 4.3) is shown in light blue. **d.** 3X3C model (compact conformation, pH 7.5-8.5) is shown in green. The hydrophobic membrane core boundaries are shown with the black lines. The cavities are colored pink. A prosthetic group retinal is colored dark green.



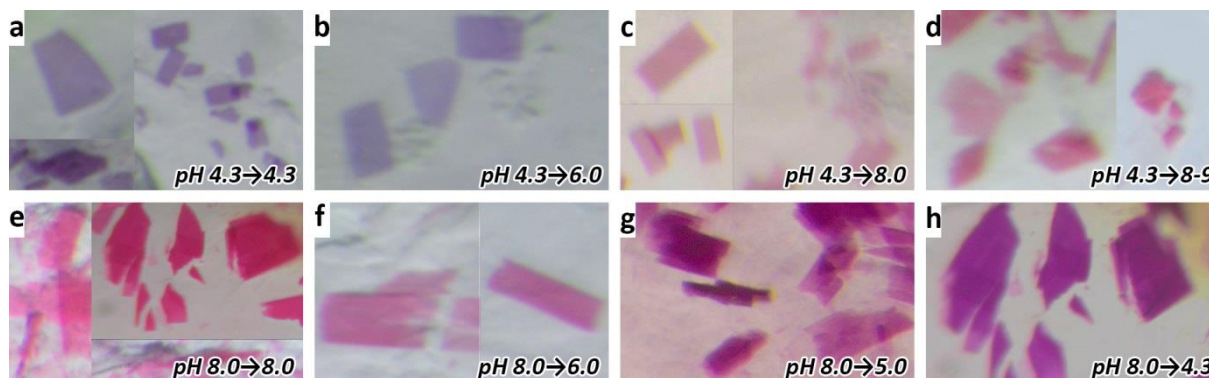
**Fig. S4. Activity tests of KR2 potassium-pumping mutants.** Pumping activity of KR2 and its mutants in *E.coli* cells suspension. The solutions contain 100 mM NaCl or KCl (black, dashed) and 100 mM NaCl or KCl and 30  $\mu\text{M}$  CCCP (black, solid). pH of the starting solutions was around 7. The cells were illuminated for 300 s (yellow area on the plots).



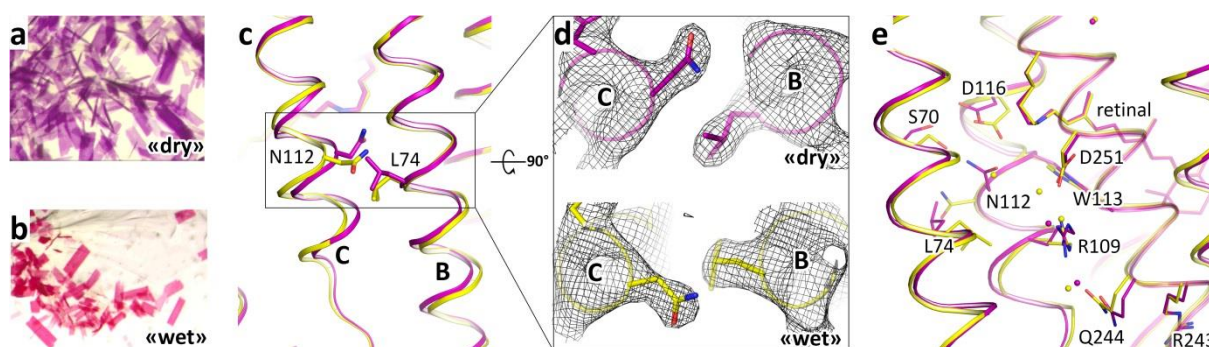
**Fig. S5. Structural alignment of potassium-pumping KR2 mutants with the model of wild-type protein.** **a.** Alignment of G263F (orange) and WT monomeric forms (PDB 4XTL, lightblue). **b.** Alignment of G263F (lightpink) and WT pentameric forms (yellow). The cavities inside WT protein are shown and colored pink. **c.** Alignment of S254A (cyan) and WT monomeric forms (PDB 4XTL, lightblue). **d.** Alignment of S254A (purple) and WT pentameric forms (yellow).



**Fig. S6. Structures of potassium-pumping KR2 mutants.** **a.** Cytoplasmic region of G263F monomeric form. **b.** Cytoplasmic region of G263F pentameric form. **c.** Retinal region of S254A monomeric form. **d.** Retinal region of S254A pentameric form. The cavities are colored pink. A prosthetic group retinal is colored dark green.

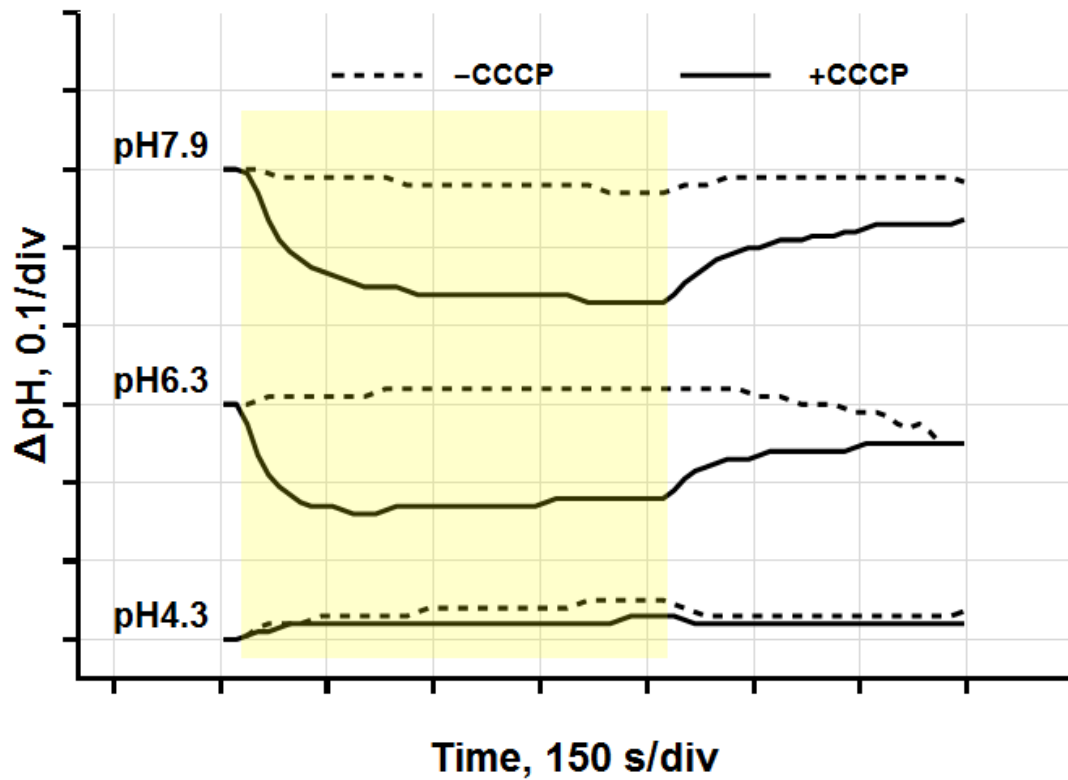


**Fig. S7. KR2 crystal soaking.** **a-d.** Crystals grown at pH 4.3, contain protein monomer in asymmetric unit. **e-h.** Crystals grown at pH 8.0, contain protein pentamer in asymmetric unit. Size of KR2 crystals, grown at pH 4.3 and 8.0 were 50-100  $\mu\text{m}$  and 100-200  $\mu\text{m}$  respectively.

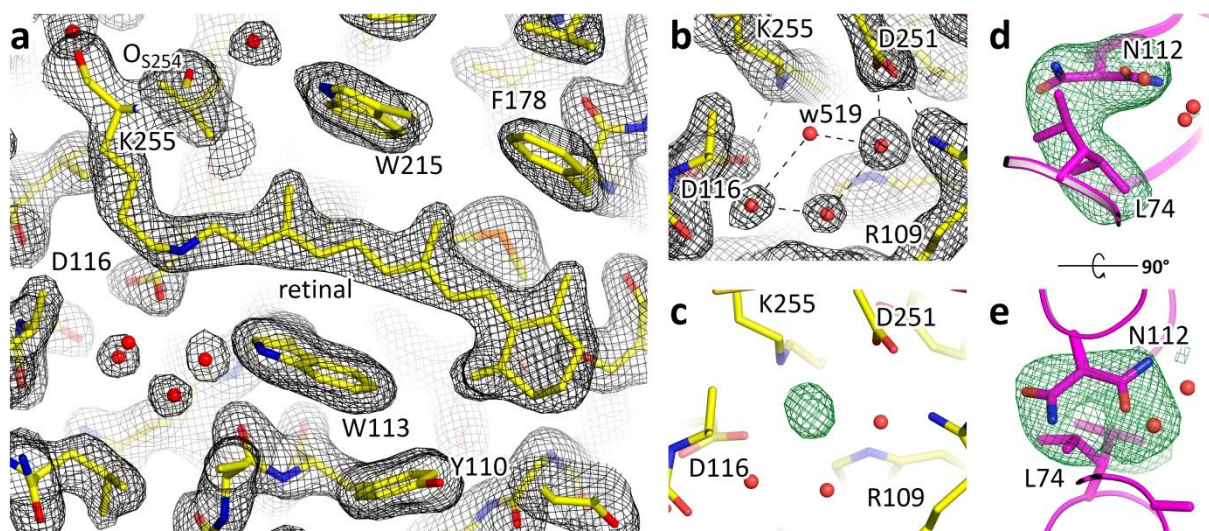


**Fig. S8. KR2 dry and wet forms.** **a.** Crystals of pentameric KR2 grown at pH 8.0, wizened and changed their color from red to purple with drying after 6 months in the crystallization plate, revealing “dry” conformation of the protein. **b.** The same wizened crystals after soaking in 3.4 M sodium malonate pH 8.0, revealing “wet” conformation of the protein. **c.** Side view of the aligned protomers (helices B and C) of the “dry” and “wet” KR2 models. Asn112 and Leu74 side chains positions are shown with sticks. **d.**  $2F_o - F_c$  electron density maps are shown for Asn112 and Leu74 side chains in “dry” and “wet” conformations. Maps are contoured at the level of  $1.5\sigma$ . **e.** Detailed view of the two protomers aligned. Key amino acids side chains in KR2 are shown with sticks. Helix A is hidden for clarity.





**Fig. S9. Activity tests of KR2 at different pH.** Pumping activity of KR2 reconstituted into lipidic vesicles. The unbuffered solutions contain 100 mM NaCl (black, dashed) and 100 mM NaCl and 30  $\mu\text{M}$  CCCP (black, solid). Starting pH was adjusted by dialysis against 100 mM NaCl unbuffered solution with needed pH. The liposomes were illuminated for 600 s (yellow area on the plots).



**Fig. S10. Electron density maps of KR2 structures.** **a.** Example of  $2F_o-F_c$  electron density map around retinal in KR2 structure at pH 8.0. The map is contoured at the level of  $1.5\sigma$ . **b.**  $2F_o-F_c$  electron density map around water molecule 519. The map is contoured at the level of  $1.5\sigma$ . Hydrogen bonds are shown with dashed black lines. **c.**  $F_o-F_c$  difference electron density map of KR2 model at pH 8.0 without Wat519 refined against experimental data. The map is contoured at the level of  $4.0\sigma$ . The density has spherical shape and is located at reasonable distances from neighbor water molecules and residues. **d, e.** Model of KR2 at pH 5.0 is shown in magenta. Difference  $F_o-F_c$  electron density map of the model at pH 5.0 without both Asn112 and Leu74 side chains refined against experimental data is shown in green mesh. The map is contoured at the level of  $3.0\sigma$ . Two conformations fit difference map accurately, however they are poorly ordered, especially Leu74, which  $2F_o-F_c$  map is shown to be weak. Two perpendicular views are shown.

**Table S1. Summary information of crystal structures obtained.**

| Protein       | pH of precipitant    | 4.3            |               |               |               | 8.0                                  |                                |                |                |
|---------------|----------------------|----------------|---------------|---------------|---------------|--------------------------------------|--------------------------------|----------------|----------------|
|               | pH of soaking buffer | 4.3            | 6.0           | 8.0           | 8.9           | 4.3                                  | 5.0                            | 6.0            | 8.0            |
| KR2 wild type | Conformation         | Compact 1.45 Å | Compact 2.3 Å | Compact 1.8 Å | Compact 2.5 Å | Bad diffraction 7.0 Å High mosaicity | Double: Expanded-compact 2.6 Å | Expanded 2.7 Å | Expanded 2.2 Å |
|               | Oligomeric state     | Monomeric      |               |               |               | Pentameric                           |                                |                |                |
| H30K          | Conformation         |                |               |               |               |                                      |                                |                |                |
|               | Oligomeric state     |                |               |               |               | -                                    |                                |                |                |
| Y154F         | Conformation         |                |               |               |               |                                      |                                |                |                |
|               | Oligomeric state     |                |               |               |               | -                                    |                                |                |                |
| G263F         | Conformation         | Compact 2.0 Å  |               |               |               |                                      |                                |                |                |
|               | Oligomeric state     | Monomeric      |               |               |               | -                                    |                                |                |                |
| S254A         | Conformation         | Compact 2.1 Å  |               |               |               |                                      |                                |                |                |
|               | Oligomeric state     | Monomeric      |               |               |               | -                                    |                                |                |                |

**Table S2. Data collection and refinement statistics of the wild-type KR2.**

|                                       | <i>Pentameric</i>            |                              |                              | <i>Monomeric</i>             |                              |                              | <i>Pentameric,</i><br><i>“dry”</i> | <i>Pentameric,</i><br><i>“wet”</i> |
|---------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------------|------------------------------------|
|                                       | pH                           | 8.0                          | 6.0                          | 5.0                          | 6.0                          | 8.0                          | 8.9                                | 8.0                                |
| <b>Data collection</b>                |                              |                              |                              |                              |                              |                              |                                    |                                    |
| Space group                           | C222 <sub>1</sub>            | C222 <sub>1</sub>            | C222 <sub>1</sub>            | I222                         | I222                         | I222                         | C222 <sub>1</sub>                  | C222 <sub>1</sub>                  |
| Cell dimensions                       |                              |                              |                              |                              |                              |                              |                                    |                                    |
| <i>a, b, c</i> (Å)                    | 131.36,<br>239.59,<br>135.25 | 131.43,<br>240.79,<br>135.74 | 130.24,<br>241.39,<br>135.11 | 40.33,<br>81.25,<br>233.36   | 40.41,<br>82.32,<br>233.35   | 40.60,<br>82.25,<br>234.18   | 128.18,<br>239.72,<br>131.91       | 130.63,<br>240.47,<br>135.61       |
| $\alpha, \beta, \gamma$ (°)           | 90, 90,<br>90                | 90, 90,<br>90                | 90, 90,<br>90                | 90, 90,<br>90                | 90, 90,<br>90                | 90, 90,<br>90                | 90, 90, 90                         | 90, 90, 90                         |
| Wavelength (Å)                        | 1.000                        | 1.000                        | 0.978                        | 1.000                        | 0.978                        | 1.000                        | 0.976                              | 0.976                              |
| Resolution (Å)                        | 48.03-2.2<br>(2.24-<br>2.20) | 48.21-2.7<br>(2.77-<br>2.70) | 48.08-2.6<br>(2.66-<br>2.60) | 40.62-2.3<br>(2.39-<br>2.30) | 40.60-1.8<br>(1.84-<br>1.80) | 40.70-2.5<br>(2.60-<br>2.50) | 47.28-3.0<br>(3.12-3.00)           | 48.13-2.8<br>(2.89-2.80)           |
| $R_{\text{merge}}$ (%)                | 13.0<br>(143.5)              | 19.0<br>(130.8)              | 8.7<br>(174.6)               | 10.5<br>(105.8)              | 3.9 (42.8)                   | 10.7<br>(153.7)              | 30.4 (147.5)                       | 12.9<br>(118.7)                    |
| $R_{\text{pim}}$ (%)                  | 1.5 (16.7)                   | 9.1 (62.7)                   | 4.1 (79.4)                   | 4.9 (50.6)                   | 1.9 (20.1)                   | 5.3 (75.0)                   | 12.7 (61.8)                        | 10.0 (91.5)                        |
| $I/\sigma I$                          | 30.8 (4.4)                   | 7.9 (1.1)                    | 9.7 (0.9)                    | 10.4 (1.5)                   | 19.1 (3.3)                   | 9.4 (0.9)                    | 5.7 (1.4)                          | 5.6 (1.2)                          |
| $CC1/2$ (%)                           | 99.6<br>(96.2)               | 99.1<br>(63.2)               | 99.9<br>(44.8)               | 99.7<br>(68.3)               | 99.9<br>(92.5)               | 99.9<br>(56.0)               | 98.9 (66.2)                        | 99.7 (78.2)                        |
| Completeness (%)                      | 100.0<br>(100.0)             | 96.9<br>(98.6)               | 98.9<br>(99.8)               | 99.7<br>(99.5)               | 99.9<br>(99.9)               | 99.6<br>(99.9)               | 99.9 (100.0)                       | 99.6 (99.4)                        |
| Unique reflections                    | 107966<br>(5398)             | 57277<br>(4469)              | 64789<br>(4559)              | 17576<br>(1841)              | 36723<br>(2179)              | 14043<br>(1557)              | 41018<br>(4562)                    | 52539<br>(4497)                    |
| <b>Refinement</b>                     |                              |                              |                              |                              |                              |                              |                                    |                                    |
| Resolution (Å)                        | 48.08-2.2                    | 48.26-2.7                    | 48.13-2.6                    | 20.00-2.3                    | 20.00-1.8                    | 20.00-2.6                    | 47.32-3.0                          | 48.17-2.8                          |
| No. reflections                       | 102,502                      | 54,451                       | 61,464                       | 16,711                       | 35,277                       | 11,878                       | 38,937                             | 49,912                             |
| $R_{\text{work}}/R_{\text{free}}$ (%) | 14.9/17.2                    | 22.3/25.0                    | 19.1/24.5                    | 20.2/25.8                    | 14.2/18.1                    | 22.1/27.1                    | 21.7/25.2                          | 18.8/22.3                          |
| No. atoms                             |                              |                              |                              |                              |                              |                              |                                    |                                    |
| Protein                               | 10886                        | 10872                        | 10910                        | 2125                         | 2247                         | 2129                         | 10753                              | 10872                              |
| Water                                 | 541                          | 316                          | 97                           | 47                           | 93                           | 13                           | 41                                 | 192                                |
| Lipid fragments                       | 1210                         | 899                          | 417                          | 275                          | 185                          | 54                           | 201                                | 895                                |
| Retinal                               | 100                          | 100                          | 100                          | 20                           | 20                           | 20                           | 100                                | 100                                |
| Sodium ions                           | 5                            | 5                            | 5                            | 1                            | 1                            | -                            | 5                                  | 5                                  |
| $B$ factors (Å <sup>2</sup> )         |                              |                              |                              |                              |                              |                              |                                    |                                    |
| Protein                               | 35.1                         | 38.3                         | 75.7                         | 43.7                         | 35.4                         | 64.3                         | 45.5                               | 62.2                               |
| Water                                 | 44.5                         | 35.2                         | 72.1                         | 50.3                         | 52.3                         | 58.3                         | 36.1                               | 55.7                               |
| Lipid fragments                       | 76.6                         | 69.0                         | 93.7                         | 78.3                         | 62.2                         | 73.6                         | 56.0                               | 95.1                               |
| Retinal                               | 28.7                         | 32.3                         | 71.1                         | 35.3                         | 30.2                         | 62.0                         | 52.3                               | 61.4                               |
| Sodium ions                           | 25.6                         | 33.1                         | 69.9                         | 58.9                         | 44.1                         | -                            | 34.9                               | 50.3                               |
| R.m.s deviations                      |                              |                              |                              |                              |                              |                              |                                    |                                    |
| Protein bond lengths (Å)              | 0.0041                       | 0.0024                       | 0.0023                       | 0.0029                       | 0.0035                       | 0.0023                       | 0.0030                             | 0.0028                             |
| Protein bond angles (°)               | 0.831                        | 0.560                        | 0.541                        | 0.584                        | 0.767                        | 0.504                        | 0.590                              | 0.600                              |

**Table S3. Data collection and refinement statistics of KR2 mutants.**

|                                       | <i>G263F,</i><br><i>monomeric</i> | <i>G263F,</i><br><i>pentameric</i> | <i>S254A,</i><br><i>monomeric</i> | <i>S254A,</i><br><i>pentameric</i> | <i>Y154F,</i><br><i>monomeric</i> | <i>H30K,</i><br><i>monomeric</i> |
|---------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|----------------------------------|
| pH                                    | 4.3                               | 8.0                                | 4.3                               | 8.0                                | 8.0                               | 8.0                              |
| <b>Data collection</b>                |                                   |                                    |                                   |                                    |                                   |                                  |
| Space group                           | I222                              | C222 <sub>1</sub>                  | I222                              | C222 <sub>1</sub>                  | I222                              | I222                             |
| Cell dimensions                       |                                   |                                    |                                   |                                    |                                   |                                  |
| <i>a, b, c</i> (Å)                    | 40.77, 81.96,<br>232.93           | 131.68, 239.68,<br>134.58          | 40.80, 83.00,<br>234.10           | 131.40, 240.04,<br>135.14          | 40.47, 81.85,<br>233.57           | 40.74, 84.18,<br>234.54          |
| $\alpha, \beta, \gamma$ (°)           | 90, 90, 90                        | 90, 90, 90                         | 90, 90, 90                        | 90, 90, 90                         | 90, 90, 90                        | 90, 90, 90                       |
| Wavelength (Å)                        | 0.976                             | 1.003                              | 0.968                             | 1.000                              | 0.972                             | 0.968                            |
| Resolution (Å)                        | 40.5-2.0 (2.05-<br>2.00)          | 47.94-2.40<br>(2.45-2.40)          | 40.78-2.1<br>(2.16-2.10)          | 48.05-2.4<br>(2.45-2.40)           | 40.93-1.8<br>(1.84-1.80)          | 42.09-2.2<br>(2.27-2.20)         |
| $R_{\text{merge}}$ (%)                | 4.8 (40.5)                        | 12.7 (167.4)                       | 8.8 (167.4)                       | 11.9 (148.1)                       | 6.1 (87.0)                        | 15.8 (170.2)                     |
| $R_{\text{pim}}$ (%)                  | 2.7 (22.6)                        | 5.3 (68.0)                         | 4.6 (86.6)                        | 6.0 (74.2)                         | 3.1 (42.8)                        | 7.3 (79.6)                       |
| $I/\sigma$                            | 14.8 (3.2)                        | 9.8 (1.3)                          | 10.9 (1.0)                        | 9.1 (1.0)                          | 13.2 (1.7)                        | 7.4 (1.0)                        |
| $CC1/2$ (%)                           | 99.9 (90.1)                       | 99.8 (71.0)                        | 99.9 (53.0)                       | 99.8 (52.1)                        | 99.8 (88.5)                       | 99.7 (47.6)                      |
| Completeness (%)                      | 99.5 (100.0)                      | 99.9 (99.9)                        | 99.4 (100.0)                      | 99.9 (99.9)                        | 99.6 (99.6)                       | 99.9 (100.0)                     |
| Unique reflections                    | 26,858 (1983)                     | 83,145 (4490)                      | 23679 (1936)                      | 83365 (4530)                       | 36488 (2168)                      | 21097 (1825)                     |
| <b>Refinement</b>                     |                                   |                                    |                                   |                                    |                                   |                                  |
| Resolution (Å)                        | 20.00-2.0                         | 47.98-2.4                          | 20.00-2.1                         | 44.91-2.4                          | 20.00-1.8                         | 20.00-2.2                        |
| No. reflections                       | 25,525                            | 78,915                             | 22,551                            | 79,117                             | 36,450                            | 20,046                           |
| $R_{\text{work}}/R_{\text{free}}$ (%) | 17.7/22.7                         | 17.2/20.5                          | 22.6/27.9                         | 18.1/21.2                          | 16.3/20.3                         | 20.9/23.8                        |
| No. atoms                             |                                   |                                    |                                   |                                    |                                   |                                  |
| Protein                               | 2161                              | 10877                              | 2126                              | 10867                              | 2239                              | 2111                             |
| Water                                 | 54                                | 462                                | 28                                | 328                                | 97                                | 38                               |
| Lipid fragments                       | 337                               | 1648                               | 291                               | 894                                | 162                               | 289                              |
| Retinal                               | 20                                | 100                                | 20                                | 100                                | 20                                | 20                               |
| Sodium ions                           | 1                                 | 5                                  | 1                                 | 5                                  | 1                                 | 1                                |
| $B$ factors (Å <sup>2</sup> )         |                                   |                                    |                                   |                                    |                                   |                                  |
| Protein                               | 39.2                              | 49.7                               | 51.4                              | 52.4                               | 32.4                              | 38.8                             |
| Water                                 | 47.0                              | 56.3                               | 48.7                              | 55.4                               | 50.4                              | 39.8                             |
| Lipid fragments                       | 80.4                              | 98.6                               | 84.0                              | 91.7                               | 56.0                              | 74.6                             |
| Retinal                               | 31.3                              | 43.4                               | 42.1                              | 48.0                               | 26.7                              | 32.2                             |
| Sodium ions                           | 42.4                              | 41.4                               | 69.6                              | 42.7                               | 37.3                              | 60.9                             |
| R.m.s deviations                      |                                   |                                    |                                   |                                    |                                   |                                  |
| Protein bond lengths (Å)              | 0.0043                            | 0.0033                             | 0.0028                            | 0.0028                             | 0.0016                            | 0.0038                           |
| Protein bond angles (°)               | 0.769                             | 0.704                              | 0.570                             | 0.628                              | 0.614                             | 0.730                            |

**Table S4. Distance between Asp<sup>116</sup> oxygen and RSB nitrogen atoms in KR2 models.**

| <b>PDB</b> | <b>pH</b> | <b>Oligomeric state</b> | <b>Distance, Å</b>            |
|------------|-----------|-------------------------|-------------------------------|
| This work  | 8.0       | Pentameric              | 2.8                           |
|            | 6.0       |                         | 2.9                           |
|            | 5.0       |                         | 3.2                           |
|            | 8.9       | Monomeric               | 3.3                           |
|            | 8.0       |                         | 3.3                           |
|            | 6.0       |                         | 3.7                           |
|            | 4.3       |                         | 3.6                           |
| 4XTO       | 5.6       | Pentameric              | 3.3-3.9 (compact)             |
| 4XTN       | 4.9       |                         | 2.9 (expanded) -3.5 (compact) |
| 4XTL       | 4.3       | Monomeric               | 3.6                           |
| 3X3B       | 4.0       |                         | 3.7                           |
| 3X3C       | 7.5-8.5   |                         | 2.5-3.3                       |