

Table S1. QM/MM calculated and experimental absorption maxima values (in nm).

Rhodopsin	RCSB Code	Dowser + +	Dowser	WaterDock	WaterDock-2.0	Crystallographic	Experiment
H. salinarum bacteriorhodopsin	5ZIM	550	540	576	525	562	568 <sup>1</sup>
H. walsbyi bacteriorhodopsin	5ITC	515	514	510	502	486	550 <sup>2</sup>
Archaerhodopsin-1	1UAZ	561	559	574	565	582	555 <sup>3</sup>
Archaerhodopsin-2	3WQJ	545	550	565	567	549	550 <sup>4</sup>
Archaerhodopsin-3	6GUX	538	553	544	542	550	552 <sup>5</sup>
Acetabularia rhodopsin 2	3AM6	518	519	492	519	490	532 <sup>6</sup>
N. pharaonis sensory rhodopsin	3QAP	464	460	488	465	468	497 <sup>7</sup>
cruxrhodopsin-3	4JR8	568	576	596	555	583	560 <sup>8</sup>
KR2 sodium pump pH 4.3	4XTL	554	571	585	559	573	566 <sup>9</sup>
E. sibiricum rhodopsin	4HYJ	525	464	469	545	512	534 <sup>10</sup>
Acetabularia rhodopsin 1	5AX0	514	472	456	472	470	520 <sup>11</sup>
channelrhodopsin-2	6EID	451	426	431	390	422	460 <sup>12</sup>
ch1ch2 chimera	3UG9	463	463	456	453	453	470 <sup>13</sup>
blue abs. proteorhodopsin	4JQ6	491	509	555	514	551	490 <sup>14</sup>
xanthorhodopsin	3DDL	540	536	597	573	531	560 <sup>15</sup>
deltarhodopsin	4FBZ	554	532	544	537	556	550 <sup>16</sup>
H. marismortui bacteriorhodopsin I D94 N	4PXK	554	494	485	520	519	567 <sup>17</sup>
thermophilic rhodopsin	5AZD	515	470	512	516	485	530 <sup>18</sup>
Viral rhodopsin OLPVRII	6SQG	525	535	518	526	535	514 <sup>19</sup>
gloebacter rhodopsin	6NWD	539	526	569	579	567	545 <sup>20</sup>
chrimson	5ZIH	568	572	575	567	588	590 <sup>21</sup>
C. subellipsoidea rhodopsin	6GYH	528	530	530	531	525	535 <sup>22</sup>
DTS-motif rhodopsin	6J00	515	495	544	518	518	509 <sup>23</sup>
GtACR1	6CSM	529	528	520	500	505	514 <sup>24</sup>

Table S1, continuation. QM/MM calculated and experimental absorption maxima values (in nm).

Rhodopsin	RCSB Code	Dowser+ +	Dowser	WaterDock	WaterDock-2.0	Crystallographic	Experiment
N. pharaonis halorhodopsin Cl-bound	3A7K	525	491	532	537	509	578 <sup>25</sup>
H. salinarum halorhodopsin Cl-bound	1E12	544	492	518	512	521	578 <sup>26</sup>
N. pharaonis halorhodopsin anion-free	5ETZ	565	588	573	574	541	600 <sup>27</sup>
Anabaena sensory rhodopsin	1XIO	536	513	516	539	549	549 <sup>28</sup>
KR2 sodium pump pH 8.0	6RF6	530	500	511	480	501	528 <sup>29</sup>
HOT75 blue-abs. proteorhodopsin D97N/Q105L	4KNF	533	530	546	544	536	520 <sup>30</sup>
48C12 heliorhodopsin pH 4.3	6SU4	550	491	468	489	483	568 <sup>31</sup>
Cl-pump CIR Cl-bound	5G28	472	479	463	477	461	530 <sup>32</sup>
iC+ +	6CSN	463	467	445	500	444	483 <sup>33</sup>
T. archaeon heliorhodopsin	6IS6	493	483	443	528	480	543 <sup>34</sup>
xenorhodopsin	6EYU	553	547	551	554	556	565 <sup>35</sup>
H. salinarum bacteriorhodopsin K	1M0K	563	555	590	536	543	590 <sup>1</sup>
H. salinarum bacteriorhodopsin L	1O0A	574	552	576	565	561	550 <sup>1</sup>
H. salinarum bacteriorhodopsin N	1P8U	540	519	560	540	491	560 <sup>1</sup>
N. pharaonis sensory rhodopsin K	2F93	520	579	537	521	534	510 <sup>7</sup>
H. salinarum halorhodopsin T203V L1 Cl-bound	2JAG	506	504	508	495	499	520 <sup>36</sup>
bovine rhodopsin	1U19	480	449	486	490	471	500 <sup>37</sup>
squid rhodopsin	2Z73	480	466	449	468	471	480 <sup>38</sup>
jumping spider rhodopsin-1	6I9K	489	457	495	480	474	505 <sup>39</sup>
bovine bathorhodopsin	2G87	514	535	452	469	504	529 <sup>40</sup>
bovine lumirhodopsin	2HPY	482	504	480	506	515	492 <sup>40</sup>
squid lumirhodopsin	4WW3	489	474	460	474	497	514 <sup>41</sup>
squid bathorhodopsin	3AYM	489	487	482	480	503	535 <sup>42</sup>
squid isorhodopsin	3AYN	447	426	425	416	441	468 <sup>42</sup>

Table S2. Protonated titratable residues in the rhodopsin QM/MM models.

Rhodopsin	RCSB Code	Protonated residues
H. salinarum bacteriorhodopsin	5ZIM	Asp85 (7.07), Asp96 (8.32), Asp115 (7.56), Glu194 (9.51)
H. walsbyi bacteriorhodopsin	5ITC	Asp93 (7.05), Asp104 (8.07), Asp123 (7.26), Glu202 (10.15)
Archaerhodopsin-1	1UAZ	Asp91 (7.26), Asp102 (7.83), Asp121 (7.27), Glu210 (11.11)
Archaerhodopsin-2	3WQJ	Asp90 (7.00), Asp101 (8.05), Asp120 (7.24), Glu209 (10.52)
Archaerhodopsin-3	6GUX	Asp95 (7.50), Asp106 (8.13), Glu214 (10.49)
Acetabularia rhodopsin 2	3AM6	Asp81 (7.95), Asp111 (7.31)
N. pharaonis sensory rhodopsin	3QAP	Asp75 (7.21)
cruxrhodopsin-3	4JR8	Asp83 (7.01), Asp94 (8.14), Asp110 (8.70), Glu205 (9.56)
KR2 sodium pump pH 4.3	4XTL	Asp116 (8.99)
E. sibiricum rhodopsin	4HYJ	His57 (7.01), Asp119 (7.39)
Acetabularia rhodopsin 1	5AX0	Asp89 (7.13)
channelrhodopsin-2	6EID	Glu90 (9.00), Glu101 (8.79), Glu123 (7.02)
ch1ch2 chimera	3UG9	Glu122 (8.51), Glu129 (8.79), Glu139 (7.81), Asp195 (7.47)
blue abs. proteorhodopsin	4JQ6	Glu90 (9.55)
xanthorhodopsin	3DDL	Glu107 (9.38)
deltarhodopsin	4FBZ	Asp95 (8.58), Glu204 (11.31)
H. marismortui bacteriorhodopsin I D94 N	4PXK	Asp113 (7.31), Glu209 (11.07)
thermophilic rhodopsin	5AZD	Glu106 (8.42)
Viral rhodopsin OLPVR II	6SQG	Glu42 (8.07), Asp74 (7.03)
gloebacter rhodopsin	6NWD	Glu132 (8.53)
chrimson	5ZIH	Glu132 (11.55), Glu143 (9.00), Glu108 (10.22), Asp295 (7.00)
C. subellipsoidea rhodopsin	6GYH	Asp86 (7.02), Asp116 (7.43), Glu193 (7.14)
DTS-motif rhodopsin	6JO0	Glu54 (10.62), Asp81 (7.06)
GtACR1	6CSM	Glu68 (9.39), Glu163 (8.57)

Table S2, continuation. Protonated titratable residues in the rhodopsin QM/MM models.

Rhodopsin	RCSB Code	Protonated residues
N. pharaonis halorhodopsin Cl-bound	3A7K	Asp156 (7.48)
H. salinarum halorhodopsin Cl-bound	1E12	Asp141 (7.09)
N. pharaonis halorhodopsin anion-free	5ETZ	Asp156 (7.45)
Anabaena sensory rhodopsin	1XIO	Glu4 (8.88), Glu36 (8.84), Glu62 (13.29), Asp198 (8.36)
KR2 sodium pump pH 8.0	6RF6	-
HOT75 blue-abs. proteorhodopsin D97N/Q105L	4KNF	-
48C12 heliorhodopsin pH 4.3	6SU4	Glu107 (7.10)
Cl-pump CIR Cl-bound	5G28	-
iC++	6CSN	-
T. archaeon heliorhodopsin	6IS6	Glu108 (7.18)
xenorhodopsin	6EYU	Asp76 (7.13)
H. salinarum bacteriorhodopsin K	1M0K	Asp85 (7.72), Asp96 (8.83), Asp115 (7.25), Glu204 (10.26)
H. salinarum bacteriorhodopsin L	1O0A	Asp85 (7.71), Asp96 (8.44), Asp115 (7.66), Glu204 (9.90)
H. salinarum bacteriorhodopsin N	1P8U	Asp96 (8.64), Asp115 (7.72), Glu204 (9.59)
N. pharaonis sensory rhodopsin K	2F93	Asp75 (8.00)
H. salinarum halorhodopsin T203V L1 Cl-bound	2JAG	Asp141 (7.11)
bovine rhodopsin	1U19	Asp83 (9.19), Glu122 (9.18), Glu181 (7.28)
squid rhodopsin	2Z73	Asp80 (7.77)
jumping spider rhodopsin-1	6I9K	Asp96 (7.15), Glu194 (7.11)
bovine bathorhodopsin	2G87	Asp83 (9.24), Glu122 (9.16), Glu181 (7.38)
bovine lumirhodopsin	2HPY	Asp83 (8.47), Glu122 (8.05), Glu181 (7.07)
squid lumirhodopsin	4WW3	Asp80 (8.10)
squid bathorhodopsin	3AYM	Asp80 (7.82)
squid isorhodopsin	3AYN	Asp80 (7.75)

Table S3. The direct effect of water molecules on QM/MM calculated  $\lambda_{max}$  values (in nm).

RCSB Code	Dowser+ +	M[Direct7Å]	M[Direct]
5ZIM	550	551	565
5ITC	515	512	530
1UAZ	561	556	583
3WQJ	545	541	566
6GUX	538	539	560
3AM6	518	514	500
3QAP	464	463	488
4JR8	568	568	583
4XTL	554	552	555
4HYJ	525	528	529
5AX0	514	508	491
6EID	451	448	446
3UG9	463	467	437
4JQ6	491	498	502
3DDL	540	538	573
4FBZ	554	550	551
4PXK	554	552	508
5AZD	515	515	488
6SQG	525	525	522
6NWD	539	539	562
5ZIH	568	571	571
6GYH	528	524	541
6JO0	515	515	526
6CSM	529	528	531
3A7K	525	525	497
1E12	544	542	515
5ETZ	565	565	573
1XIO	536	533	532
6RF6	530	533	479
4KNF	533	534	541
6SU4	550	555	588
5G28	472	474	459
6CSN	463	465	462
6IS6	493	498	490
6EYU	553	554	561
1M0K	563	564	576
1O0A	574	574	582
1P8U	540	535	525
2F93	520	518	525
2JAG	506	507	499
1U19	480	476	462
2Z73	480	479	476
6I9K	489	489	503
2G87	514	520	499
2HPY	482	484	471
4WW3	489	488	470
3AYM	489	487	488
3AYN	447	446	441

### Dowser and Dowser++ parameter set for the retinal protonated Schiff base (atomdict.db file).

RESIDUE REL TERM NH3 COO  
ATOM REL N C CA 1.320 114.0 180.0 -0.280 N  
ATOM REL H N NOT 1.000 123.0 0.0 0.280 H  
ATOM REL CA N C 1.470 123.0 180.0 0.000 CH1  
ATOM REL CB CA CG 1.530 110.0 60.0 0.000 CH2  
ATOM REL CG CB CD 1.530 112.0 180.0 0.000 CH2  
ATOM REL CD CG CE 1.530 112.0 180.0 0.000 CH2  
ATOM REL CE CD NZ 1.530 112.0 180.0 0.450 CH2  
ATOM REL NZ CE HZ1 1.470 112.0 180.0 0.380 N  
ATOM REL HZ1 NZ NOT 1.000 109.5 180.0 -0.400 H  
ATOM REL C15 NZ C14 1.280 125.6 180.0 0.570 CH1  
ATOM REL C14 C15 C13 1.470 123.0 0.0 0.000 CH1  
ATOM REL C13 C14 C12 1.400 123.0 180.0 0.000 CR  
ATOM REL C20 C13 NOT 1.520 120.0 180.0 0.000 CH3  
ATOM REL C12 C13 C11 1.400 123.0 180.0 0.000 CH1  
ATOM REL C11 C12 C10 1.370 123.0 180.0 0.000 CH1  
ATOM REL C10 C11 C9 1.400 123.0 180.0 0.000 CH1  
ATOM REL C9 C10 C8 1.370 123.0 180.0 0.000 CR  
ATOM REL C19 C9 NOT 1.504 120.0 0.0 0.000 CH3  
ATOM REL C8 C9 C7 1.470 123.5 -180.0 0.000 CH1  
ATOM REL C7 C8 C6 1.340 123.5 180.0 0.000 CH1  
ATOM REL C6 C7 C1 1.470 123.5 180.0 0.000 CR  
ATOM REL C1 C6 C2 1.500 112.2 0.0 0.000 CR  
ATOM REL C16 C1 NOT 1.538 112.2 -60.0 0.000 CH3  
ATOM REL C17 C1 NOT 1.538 112.2 -60.0 0.000 CH3  
ATOM REL C5 C6 C4 1.340 123.5 180.0 0.000 CR  
ATOM REL C18 C5 NOT 1.500 123.5 0.0 0.000 CH3  
ATOM REL C4 C5 C3 1.500 112.0 0.0 0.000 CH2  
ATOM REL C3 C4 C2 1.530 113.6 -60.0 0.000 CH2  
ATOM REL C2 C3 C1 1.530 113.5 60.9 0.000 CH2  
ATOM REL C CA N 1.530 110.0 180.0 0.380 CR  
ATOM REL O C NOT 1.240 121.0 0.0 -0.380 O

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