Molecular Basis for Variations in the Sensitivity of Pathogenic Rhodopsin Variants to 9-cis-Retinal

Francis J. Roushar,† Andrew G. McKee,† Charles P. Kuntz,† Joseph T. Ortega, Wesley D. Penn,1 Hope Woods,3,4 Laura M. Chamness,1 Victoria Most,5 Jens Meiler,3,5 Beata Jastrzebska,2 and Jonathan P. Schlebach1*

1 Department of Chemistry, Indiana University, Bloomington, Indiana, USA
2 Department of Pharmacology, Case Western Reserve University, Cleveland, OH, USA
3 Department of Chemistry, Vanderbilt University, Nashville, TN, USA
4 Chemical and Physical Biology Program, Vanderbilt University, Nashville, TN, USA
5 Institute for Drug Development, Leipzig University, Leipzig, SAC, Germany

Keywords: Membrane Protein Folding, Rhodopsin, Proteostasis, Corrector, GPCR

† Authors contributed equally
* Corresponding author E-mail address: jschleba@indiana.edu

Contents:
- Figure S1
- Table S1
Figure S1. Thermodynamic Relationship Between Ligand Binding on the Equilibrium Fraction of Folded Protein. Equation 5 was used to simulate the impact of ligand binding on the equilibrium fraction of folded protein as a function of the ratio of ligand to its equilibrium dissociation constant. The equilibrium fraction of folded protein in the presence of ligand is plotted as a function of corresponding equilibrium fraction of folded apoprotein. This simulation reveals how the degree of rescue offered by a corrector should relate to the thermodynamic stability of the variant (x-coordinate), the concentration of the ligand, and its binding affinity.
| Mutation | Prior Classification | DMS Classification | Surface Immunostaining Intensity (Apo)* | Surface Immunostaining Intensity (+Ret)* |
|----------|----------------------|-------------------|----------------------------------------|----------------------------------------|
| T4K      | Class 2\(^1,2\)      | Non-Class 2       | 21459 ± 1373                           | 19838 ± 3633                           |
| P12R     | Unclassified         | Moderate Class 2  | 3044 ± 30                              | 5245 ± 834                             |
| N15S     | Class 2\(^1\)        | Severe Class 2    | 2058 ± 88                              | 2473 ± 303                             |
| T17M     | Class 2\(^1,3\)      | Severe Class 2    | 2211 ± 75                              | 2604 ± 227                             |
| V20G     | Class 2\(^1\)        | Severe Class 2    | 2251 ± 91                              | 2774 ± 268                             |
| P23H     | Class 2\(^1,3\)      | Severe Class 2    | 1716 ± 7                               | 2133 ± 171                             |
| P23L     | Class 2\(^1\)        | Severe Class 2    | 1585 ± 55                              | 1998 ± 255                             |
| P23A     | Class 2\(^1\)        | Severe Class 2    | 2295 ± 24                              | 2920 ± 275                             |
| Q28H     | Class 2\(^1,2\)      | Severe Class 2    | 1905 ± 82                              | 2236 ± 102                             |
| Q28R     | Class 2\(^1\)        | Severe Class 2    | 1826 ± 51                              | 2203 ± 117                             |
| M39R     | Class 4\(^1\)        | Moderate Class 2  | 7406 ± 61                              | 8483 ± 646                             |
| M44T     | Class 5\(^1\)        | Non-Class 2       | 20520 ± 250                            | 22775 ± 1128                           |
| F45L     | Class 7\(^1\)        | Non-Class 2       | 21466 ± 261                            | 23900 ± 1331                           |
| L46R     | Unclassified         | Severe Class 2    | 1575 ± 55                              | 1887 ± 86                              |
| L47R     | Class 2\(^4\)        | Moderate Class 2  | 2891 ± 92                              | 5580 ± 159                             |
| G51R     | Class 2\(^1,2,4\)    | Moderate Class 2  | 6840 ± 236                             | 11251 ± 1695                           |
| G51A     | Unclassified         | Non-Class 2       | 19022 ± 278                            | 21907 ± 945                            |
| G51V     | Class 2\(^1,2\)      | Non-Class 2       | 19866 ± 375                            | 21197 ± 624                            |
| F52Y     | Unclassified         | Moderate Class 2  | 12693 ± 203                            | 16135 ± 2076                           |
| P53R     | Class 2\(^1,2\)      | Severe Class 2    | 1659 ± 75                              | 3510 ± 247                             |
| F56Y     | Unclassified         | Non-Class 2       | 16591 ± 109                            | 18485 ± 1691                           |
| L57R     | Class 2\(^4\)        | Moderate Class 2  | 2956 ± 128                             | 4650 ± 184                             |
| T58R     | Class 2\(^1,3,4\)    | Non-Class 2       | 17889 ± 209                            | 22138 ± 1739                           |
| R69H     | Unclassified         | Non-Class 2       | 20184 ± 589                            | 23143 ± 999                            |
| del N73  | Unclassified         | Moderate Class 2  | 4913 ± 293                             | 11881 ± 2355                           |
| N78I     | Class 2\(^4\)        | Moderate Class 2  | 3185 ± 274                             | 4998 ± 625                             |
| V87L     | Unclassified         | Non-Class 2       | 18186 ± 779                            | 22577 ± 1112                           |
| V87D     | Class 2\(^1,3\)      | Moderate Class 2  | 3731 ± 472                             | 5216 ± 642                             |
| L88P     | Class 2\(^4\)        | Moderate Class 2  | 7799 ± 124                             | 12400 ± 2582                           |
| G89D     | Class 2\(^1,4\)      | Moderate Class 2  | 10580 ± 514                            | 9423 ± 1590                            |
| G90D     | Class 6\(^1\)        | Non-Class 2       | 21297 ± 242                            | 20572 ± 503                            |
| G90V     | Class 6\(^1\)        | Non-Class 2       | 21101 ± 268                            | 20872 ± 1098                           |
| T94I     | Class 6\(^1\)        | Non-Class 2       | 25614 ± 185                            | 24016 ± 497                            |
| V104I    | Unclassified         | Non-Class 2       | 20746 ± 333                            | 23099 ± 1134                           |
| V104F    | Unclassified         | Non-Class 2       | 17108 ± 351                            | 18678 ± 550                            |
| G106R    | Class 2\(^1,2\)      | Moderate Class 2  | 6597 ± 210                             | 8280 ± 1114                            |
| G106W    | Class 2\(^1,3\)      | Severe Class 2    | 2101 ± 99                              | 2993 ± 315                             |
| G109R    | Class 2\(^4\)        | Moderate Class 2  | 14723 ± 150                            | 13588 ± 2744                           |
| C110R    | Class 2\(^1\)        | Severe Class 2    | 1645 ± 73                              | 2062 ± 114                             |
| C110Y    | Class 2\(^1,2\)      | Severe Class 2    | 1660 ± 86                              | 2036 ± 96                              |
| C110F    | Class 2\(^1\)        | Severe Class 2    | 1571 ± 80                              | 1920 ± 127                             |
| G114D    | Unclassified         | Severe Class 2    | 1663 ± 103                             | 1958 ± 81                              |
| G114V    | Unclassified         | Severe Class 2    | 1575 ± 41                              | 2544 ± 155                             |
| E122G    | Unclassified         | Non-Class 2       | 21147 ± 314                            | 22740 ± 1211                           |
| Mutation | Prior Classification | DMS Classification | Surface Immunostaining Intensity (Apo)* | Surface Immunostaining Intensity (+Ret)* |
|----------|----------------------|--------------------|----------------------------------------|----------------------------------------|
| L125R    | Class 2\(^{1}\)     | Non-Class 2        | 19889 ± 220                            | 18451 ± 1729                          |
| S127F    | Unclassified         | Moderate Class 2    | 2693 ± 178                             | 3591 ± 326                            |
| L131P    | Unclassified         | Severe Class 2      | 1882 ± 130                             | 3211 ± 325                            |
| R135P    | Class 3\(^{1}\)     | Moderate Class 2    | 8559 ± 202                             | 13039 ± 2177                          |
| R135L    | Class 2\(^{2}\)     | Moderate Class 2    | 4085 ± 130                             | 7931 ± 378                            |
| R135G    | Class 3\(^{1}\)     | Moderate Class 2    | 8791 ± 181                             | 14820 ± 2169                          |
| R135W    | Class 2\(^{2}\)     | Severe Class 2      | 2129 ± 65                              | 7701 ± 299                            |
| V137M    | Class 5\(^{1}\)     | Non-Class 2         | 20724 ± 216                            | 22958 ± 621                           |
| C140S    | Unclassified         | Non-Class 2         | 20898 ± 309                            | 23562 ± 1372                          |
| E150K    | Unclassified         | Non-Class 2         | 20855 ± 324                            | 23140 ± 993                           |
| T160T    | Unclassified         | Non-Class 2         | 20350 ± 359                            | 22847 ± 1185                          |
| W161R    | Class 2\(^{1}\)     | Severe Class 2      | 1812 ± 54                              | 2330 ± 231                            |
| A164E    | Class 2\(^{1}\)     | Severe Class 2      | 1603 ± 106                             | 2606 ± 219                            |
| A164V    | Class 2\(^{1}\)     | Non-Class 2         | 18661 ± 308                            | 20327 ± 713                           |
| C167R    | Class 2\(^{1,2,4}\) | Non-Class 2         | 20651 ± 320                            | 23214 ± 1035                          |
| C167W    | Class 2\(^{1,4}\)   | Severe Class 2      | 1904 ± 28                              | 3093 ± 197                            |
| A169P    | Class 2\(^{1}\)     | Severe Class 2      | 1475 ± 67                              | 2386 ± 119                            |
| P170R    | Unclassified         | Severe Class 2      | 1461 ± 10                              | 1926 ± 283                            |
| P171E    | Unclassified         | Severe Class 2      | 1738 ± 91                              | 2465 ± 117                            |
| P171L    | Class 2\(^{1}\)     | Severe Class 2      | 1675 ± 74                              | 2429 ± 123                            |
| G174S    | Unclassified         | Non-Class 2         | 19427 ± 265                            | 23140 ± 82                            |
| S176F    | Unclassified         | Severe Class 2      | 1649 ± 36                              | 2045 ± 212                            |
| Y178N    | Class 2\(^{1}\)     | Severe Class 2      | 1622 ± 73                              | 2916 ± 178                            |
| Y178D    | Class 2\(^{1}\)     | Severe Class 2      | 1604 ± 83                              | 2142 ± 38                             |
| I179F    | Class 2\(^{2,4}\)   | Moderate Class 2    | 4667 ± 55                              | 7646 ± 964                            |
| P180A    | Class 2\(^{1}\)     | Moderate Class 2    | 3883 ± 159                             | 6555 ± 480                            |
| P180S    | Class 2\(^{1}\)     | Severe Class 2      | 1667 ± 76                              | 2127 ± 123                            |
| E181K    | Class 2\(^{1,2}\)   | Severe Class 2      | 1705 ± 42                              | 2236 ± 25                             |
| G182S    | Class 2\(^{1}\)     | Moderate Class 2    | 3154 ± 213                             | 5613 ± 471                            |
| Q184P    | Unclassified         | Severe Class 2      | 1852 ± 41                              | 2236 ± 126                            |
| C185R    | Class 2\(^{1}\)     | Severe Class 2      | 1816 ± 28                              | 2271 ± 319                            |
| S186P    | Class 2\(^{2}\)     | Severe Class 2      | 1582 ± 51                              | 1991 ± 137                            |
| S186W    | Class 2\(^{4}\)     | Non-Class 2         | 27766 ± 317                            | 28230 ± 875                           |
| C187Y    | Class 2\(^{1}\)     | Severe Class 2      | 1876 ± 64                              | 2166 ± 54                             |
| G188R    | Class 2\(^{1,2}\)   | Severe Class 2      | 1660 ± 66                              | 2113 ± 289                            |
| D190N    | Class 2\(^{1}\)     | Moderate Class 2    | 4182 ± 24                              | 5586 ± 797                            |
| D190G    | Class 2\(^{1,3}\)   | Severe Class 2      | 1788 ± 76                              | 2442 ± 115                            |
| D190Y    | Class 2\(^{1,2}\)   | Severe Class 2      | 1757 ± 98                              | 2216 ± 130                            |
| Y191C    | Unclassified         | Non-Class 2         | 17171 ± 396                            | 22547 ± 458                           |
| T193M    | Class 2\(^{4}\)     | Moderate Class 2    | 12826 ± 604                            | 17646 ± 1170                          |
| P196T    | Unclassified         | Non-Class 2         | 19201 ± 404                            | 22146 ± 1183                          |
| I205S    | Unclassified         | Non-Class 2         | 16477 ± 104                            | 19163 ± 538                           |
| M207K    | Unclassified         | Non-Class 2         | 18429 ± 352                            | 17930 ± 1375                          |
| M207R    | Class 2\(^{4}\)     | Non-Class 2         | 15790 ± 243                            | 13919 ± 804                           |
| Mutation | Prior Classification | DMS Classification | Surface Immunostaining Intensity (Apo)* | Surface Immunostaining Intensity (+Ret)* |
|----------|----------------------|-------------------|----------------------------------------|-----------------------------------------|
| V209M    | Class 7†             | Non-Class 2       | 18653 ± 98                             | 21664 ± 690                             |
| V210F    | Unclassified         | Non-Class 2       | 19755 ± 141                            | 22284 ± 776                             |
| H211R    | Class 2†             | Severe Class 2    | 1842 ± 5                               | 2237 ± 181                             |
| I214N    | Class 2†             | Non-Class 2       | 17234 ± 484                            | 19082 ± 1133                           |
| P215T    | Unclassified         | Severe Class 2    | 1521 ± 78                              | 1972 ± 215                             |
| P215L    | Unclassified         | Severe Class 2    | 2307 ± 112                             | 3037 ± 207                             |
| M216K    | Class 2†             | Non-Class 2       | 17663 ± 243                            | 20170 ± 705                            |
| M216L    | Unclassified         | Non-Class 2       | 21093 ± 31                             | 23273 ± 1180                           |
| F220C    | Class 7†             | Non-Class 2       | 19781 ± 348                            | 22066 ± 605                            |
| R252P    | Unclassified         | Severe Class 2    | 18510 ± 536                            | 20364 ± 182                            |
| del I255 | Unclassified         | Severe Class 2    | 1936 ± 36                              | 2844 ± 182                             |
| del C264 | Unclassified         | Severe Class 2    | 1994 ± 106                             | 2291 ± 53                              |
| G284S    | Unclassified         | Moderate Class 2  | 13474 ± 730                            | 14941 ± 2034                           |
| T289P    | Unclassified         | Moderate Class 2  | 2357 ± 197                             | 4688 ± 301                             |
| A295V    | Class 6†             | Non-Class 2       | 21819 ± 280                            | 22891 ± 1079                           |
| S297R    | Unclassified         | Severe Class 2    | 1638 ± 41                              | 2208 ± 185                             |
| A298D    | Unclassified         | Non-Class 2       | 17093 ± 306                            | 20131 ± 2116                           |
| K311E    | Unclassified         | Non-Class 2       | 19549 ± 358                            | 21928 ± 1173                           |
| T320N    | Unclassified         | Non-Class 2       | 20522 ± 116                            | 22270 ± 1060                           |
| L328P    | Class 1†             | Non-Class 2       | 24656 ± 401                            | 28184 ± 1242                           |
| A333V    | Unclassified         | Moderate Class 2  | 12407 ± 392                            | 14555 ± 433                            |
| E341K    | Class 2†             | Non-Class 2       | 16622 ± 95                             | 17258 ± 2084                           |
| T342M    | Class 1†             | Non-Class 2       | 24656 ± 401                            | 28184 ± 1242                           |
| S343C    | Unclassified         | Non-Class 2       | 19372 ± 323                            | 21627 ± 993                            |
| Q344P    | Class 1†             | Non-Class 2       | 17471 ± 257                            | 18834 ± 1964                           |
| Q344R    | Class 1†             | Non-Class 2       | 19609 ± 277                            | 22090 ± 1221                           |
| V345M    | Class 1†             | Non-Class 2       | 21159 ± 313                            | 23618 ± 1023                           |
| V345L    | Class 1†             | Non-Class 2       | 20578 ± 243                            | 23057 ± 1079                           |
| A346P    | Class 1†             | Non-Class 2       | 20301 ± 335                            | 22845 ± 1338                           |
| P347Q    | Class 1†             | Non-Class 2       | 20697 ± 270                            | 23161 ± 1184                           |
| P347R    | Class 1†             | Non-Class 2       | 19849 ± 272                            | 22682 ± 1304                           |
| P347L    | Class 1†             | Non-Class 2       | 22253 ± 148                            | 24541 ± 869                            |
| P347A    | Class 1†             | Non-Class 2       | 18548 ± 679                            | 21170 ± 753                            |
| P347S    | Class 1†             | Non-Class 2       | 20830 ± 291                            | 23864 ± 986                            |

[1] Athanasiou, D., Aguila, M., Bellingham, J., Li, W., McCulley, C., Reeves, P. J., and Cheetham, M. E. (2018) The molecular and cellular basis of rhodopsin retinitis pigmentosa reveals potential strategies for therapy, *Progress in Retinal and Eye Research* 62, 1-23.

[2] Kaushal, S., and Khorana, H. G. (1994) Structure and function in rhodopsin. 7. Point mutations associated with autosomal dominant retinitis pigmentosa, *Biochemistry* 33, 6121-6128.

[3] Sung, C. H., Schneider, B. G., Agarwal, N., Papermaster, D. S., and Nathans, J. (1991) Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa, *Proceedings of the National Academy of Sciences* 88, 8840.

[4] Wan, A., Place, E., Pierce, E. A., and Comander, J. (2019) Characterizing variants of unknown significance in rhodopsin: A functional genomics approach, *Human Mutation* 40, 1127-1144.