**Figure 6—source data 2. Analysis of the 14 unsuccessful predictions of A2AR CAMs/CIMs.** ΔStability (>0 means destabilized; <0 means stabilized) is the change of receptor stability when a mutation was introduced, calculated by Residue Scanning module in BioLuminate193. WT, wild-type.

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| --- | --- | --- | --- | --- | --- | --- |
| Position | Mutation | Effect on inactive state (3EML) | Effect on active state (5G53) | Prediction | Experiment result | Discussion |
| **Unsuccessful prediction of 9 CAMs** | | | | | | |
| 3×46 | I98N | ΔStability >0 | ΔStability >0, H-bonds with F442×42 | Stabilizes active state | Low expression | May affect receptor folding or trafficking. |
| 3×46 | I98E | ΔStability >0 | ΔStability >0, salt bridge with R1023×50 | Stabilizes active state | CIM, >20-fold decrease in EC50 | May affect G protein coupling interface |
| 3×49 | D101S | ΔStability >0 | ΔStability <0, H-bonds with Y11234×53 | Breaks the restrains with R1023×50 | Close to WT | May affect G protein coupling interface |
| 3×50 | R102H | ΔStability >0 | ΔStability >0, salt bridge with D101×49 | Stabilizes active state | Close to WT | May affect G protein coupling interface |
| 3×51 | Y103E | ΔStability >0, salt bridge w/R1073×55 | ΔStability >0, salt bridge with R1995×60 | Stabilizes TM5-TM6 contacts | Close to WT | May have indirect impact or no effect on TM6 rotation |
| 6×40 | I238Q | ΔStability >0 | ΔStability >0, H-bonds with R1023×50 and R2917×56 | Stabilizes active state | Close to WT | Increases TM3-TM6 contacts, but may not affect the rotation of TM6 |
| 6×40 | I238E | ΔStability >0 | ΔStability >0, salt bridge with R1023×50 | Stabilizes active state | Close to WT | Increases TM3-TM6 contacts, but may not affect the rotation of TM6 |
| 6×40 | I238A | ΔStability >0, less hydrophobic contacts | ΔStability >0, less hydrophobic contacts | Loosens TM3-TM6 contacts | Close to WT | Destabilizes both inactive and active states, but may not affect the rotation of TM6 |
| 7×45 | N280S | ΔStability >0 | ΔStability >0, H-bonds with W2466×48 | Stabilizes active state | Close to WT | Destabilizes both inactive and active states, but may not affect the rotation of TM6 |
| **Unsuccessful prediction of 5 CIMs** | | | | | | |
| 3×40 | I92Y | ΔStability >0, stacking w/F2426×44 | ΔStability >0, side chains rotate away from F2426×44 | Tightens TM3-6 contacts | Close to WT, slightly high basal activity | Makes the rotation of the cytoplasmic end of TM6 easier in active state |
| 3×50 | R102A | ΔStability >0 | ΔStability >0, affect G protein coupling interface | Reduces interaction with G protein | Close to WT | A1023×50 doesn’t affect G protein coupling for A2AR |
| 6×40 | I238M | ΔStability >0, more hydrophobic contacts | ΔStability <0, more hydrophobic contacts | Tightens TM3-6 contacts | Close to WT | May stabilize the active state, but may not affect the rotation of TM6 |
| 6×44 | F242T | ΔStability >0 | ΔStability >0, may greatly affect signal initiation | May block the rotation of TM6 | Close to WT | T2426×44 doesn’t affect signal initiation for A2AR |
| 6×44 | F242L | ΔStability >0 | ΔStability >0, may greatly affect signal initiation | May block the rotation of TM6 | Close to WT | L2426×44 doesn’t affect signal initiation for A2AR |