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**Molecular signature for receptor engagement in the metabolic peptide hormone amylin**

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*Running title*: Amylin receptor engagement

**Supplementary Modelling (SM) Tables (not submitted to Journal).**

**Supplementary modelling Table SM1.**

Hydrogen bonds and general contacts established between amylin *C*-terminus residues and the AMY1 receptor in presence (RAMP1) and without RAMP1 (NO RAMP1). Data are from 12 SuMD simulation replicas performed on amylin in its amidic (T37-NH2) *C*-terminus form. Percentages refer to the total number of frames of joint MD replicas. (sb): hydrogen bond involving an amylin side chain and the backbone of the CTR (or RAMP1); (bb): hydrogen bond involving the amylin backbone and the backbone of the CTR (or RAMP1); (bs): hydrogen bond involving amylin backbone and a CTR side chain (or RAMP1); if not specified, hydrogen bond refers to both the amylin and the receptor side chains. Only values higher than 3% for hydrogen bonds and 15% for general contacts are reported.

|  |  |
| --- | --- |
| **Amylin****Residue** | **Y37-NH2** |
| **RAMP1** | **NO RAMP1** |
| **Amy approach states** | **Metastable C terminus states** | **Amy approach states** | **Metastable C terminus states** |
| **I26** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L27** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S28** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S29** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T30** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | R126 18.5% | / | / |
| **RAMP H bonds** | / | E78 5.7%  |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N31** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | R126 22.9%N124 15.2% | / | R45 17.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **V32** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | R126 29.2% | Q52 15.2% | R45 22.1%M49 18.6%Q52 15.7% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | A87 16.9%R91 16.8%Q28 15.1% | / |  |  |
| **G33** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | A87 15.6% | / |  |  |
| **S34** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | V117 15.5% | Y131 15.6% | / | / |
| **RAMP H bonds** | / | E78 3.5% |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N35** | **CTR H bonds** | / | Y131 5.7% |  | / |
| **CTR Contacts** | N125 21.4% | W79 27.1%Y131 26.7%F99 15.6%T132 15.3% | / | W79 29.6% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | Q28 15.9% | / |  |  |
| **T36** | **CTR H bonds** |  | W118 4.3% (sb)T127 4.0%  | / | / |
| **CTR Contacts** | N125 19.5%K120 18.4%T127 16.2%V117 16.0%W118 15.6% | R126 29.0%Y131 23.0%W79 20.8% | / | W79 29.1%G78 19.8%Y131 18.3%D77 17.4%W128 15.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **Y37** | **CTR H bonds** | N125 8.5% (bb)T127 4.0% (bb) | T127 3.9% (bb)N125 3.7% (bb) | / | S129 8.0% (bb)S129 5.4% (sb)W118 3.6% (bb) |
| **CTR Contacts** | R126 32.7%T127 22.6%N124 21.2%N125 18.7%W79 16.4% | W79 62.8%Y131 45.5%F102 33.8%R126 32.2%F99 28.9%T127 28.4%S129 24.6%H121 16.1%N124 15.0% | R126 18.1%T127 17.9% | W128 50.3%Y131 47.5%T127 45.3%S129 42.2%R126 36.8%D77 30.8%G78 26.4% |
| **RAMP H bonds** | / | W84 3.9% (sb) |  |  |
| **RAMP Contacts** | F83 30.0%C82 17.8%T24 17.7%N86 15.1% | W84 23.1%P85 17.8%F83 16.8% |  |  |

**Supplementary modelling Table SM2.**

Hydrogen bonds and general contacts established between amylin *C*-terminus residues and CTR in presence (RAMP1) and without RAMP1 (NO RAMP1). Data are from three SuMD simulation replicas performed on amylin in its free carboxylic (T37-COO-) *C*-terminus forms. Percentages refer to the total number of frames of joint MD replicas. (sb): hydrogen bond involving an amylin side chain and the backbone of the CTR (or RAMP1); (bb): hydrogen bond involving the amylin backbone and the backbone of the CTR (or RAMP1); (bs): hydrogen bond involving amylin backbone and a CTR side chain (or RAMP1); if not specified, hydrogen bond refers to both the amylin and the receptor side chains. Only values higher than 5% for hydrogen bonds and 25% for general contacts are reported.

|  |  |
| --- | --- |
| **Amylin****Residue** | **Y37-COO-** |
| **RAMP1** | **NO RAMP1** |
| **Amy approach states** | **Metastable C terminus states** | **Amy approach states** | **Metastable C terminus states** |
| **I26** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | L40 21.5%F99 20.8%Y41 20.7% | L40 31.6%Y41 29.6% | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L27** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | K120 21.3%N125 21.0% | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S28** | **CTR H bonds** | D101 5.8% | D101 13.6%  | / | / |
| **CTR Contacts** | F99 21.2% | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S29** | **CTR H bonds** | D101 2.2% | D101 5.8% (bs) | / | E123 5.0% (bb) |
| **CTR Contacts** | F102 22.5%Y131 17.1%W79 10.1% | / | V117 25.9% | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | W74 26.3% |  |  |
| **T30** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N31** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | N124 17.3%R126 11.2%H121 10.8% | / | N73 23.8% | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **V32** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | M59 28.0%Q60 26.3% | N124 32.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **G33** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | / | / | Y56 31.3% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S34** | **CTR H bonds** | / | / | T75 10.2% (bs)R74 6.3% (bb)W76 5.0% (bb) | T75 7.3% (bs)W76 6.6% (bb) |
| **CTR Contacts** | / | / | T75 52.1%W76 41.5%M59 35.9%Y56 33.2%R74 24.5%N73 18.8% | W76 53.6%T75 33.3%Y56 33.2%M5930.8% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N35** | **CTR H bonds** | Y131 2.2% (sb) | / | Y56 8.6% | / |
| **CTR Contacts** | N124 12.6%E123 12.1%Y131 10.7% | F99 26.5%T131 26.0% | W76 43.9%T75 33.0%Y56 29.2% | W76 57.9%G78 50.0%D77 44.5%W79 30.1%T75 29.4%Y56 26.5% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T36** | **CTR H bonds** | / | / | T75 18.7%W76 3.7% | T75 16.6% |
| **CTR Contacts** | N124 21.9% | Y131 38.5%W79 33.1% | T127 55.8%T75 38.6%W118 37.8%W76 34.4%D77 31.7%K120 14.0% | T127 89.0%D77 39.6%W76 39.0%R126 38.7%T75 38.1%W128 32.8%W118 32.3%N124 28.3% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **Y37** | **CTR H bonds** | R126 30.7% | R126 32.4%W79 5.0% | T127 35.7%T127 28.2% (sb)R126 27.3%K120 8.7% | R126 55.6%T127 50.8%T127 42.4% (sb) |
| **CTR Contacts** | R126 62.5%N124 58.5%E123 12.7% | R126 94.8%N124 56.0%H121 42.4%T127 40.7%W128 28.3% | T127 73.7%R126 73.1%K120 31.3% | R126 99.4%T127 92.6%D77 50.0%G78 40.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | F83 36.5%T24 28.4% | / |  |  |

**Supplementary modelling Table SM3.**

Hydrogen bonds and general contacts established between amylin and CTR in presence (RAMP1) and without the RAMP1 (NO RAMP1). Data are from three MD and metadynamics simulations performed on amylin in its amidic (T37-NH2) form. Percentages refer to the total number of frames of joint MD replicas. (sb): hydrogen bond involving an amylin side chain and the backbone of the CTR (or RAMP1); (bb): hydrogen bond involving the amylin backbone and the backbone of the CTR (or RAMP1); (bs): hydrogen bond involving amylin backbone and a CTR side chain (or RAMP1); if not specified, hydrogen bond refers to both the amylin and the receptor side chains. Only values higher than 5% for hydrogen bonds and 25% for general contacts are reported*.*

|  |  |
| --- | --- |
| **Amylin****Residue** | **Y37-NH2** |
| **RAMP1** | **NO RAMP1** |
| **Classic MD****Bound state** | **Metadynamics****Bound state** | **Classic MD****Bound state** | **Metadynamics****Bound state** |
| **K1** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | L298 74.5%E294 58.2%T295 43.3%H302 43.0%V293 30.3% | T295 52.1%E294 25.6% | T295 37.4%E294 43.0%R362 30.4%V293 29.0%L298 28.8%S292 26.5% | L298 31.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N3** | **CTR H bonds** | Y234 5.8% | / | / | / |
| **CTR Contacts** | H302 95.3%M306 68.6%M230 51.0%V305 46.3%Y234 36.9% | T295 62.8%L298 59.1%E294 32.6%H302 30.1% | H302 67.6%Y299 53.3%L298 45.6%M306 37.7% | H302 35.5%L298 31.6% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T4** | **CTR H bonds** | T234 10.1% | / | / | / |
| **CTR Contacts** | F356 97.2%M306 62.0%P360 54.9%W361 49.6%Y234 31.9%M376 29.7%I380 27.3% | W361 48.5%R362 41.9%P360 34.5% | M306 62.4%F356 61.5%V357 56.8%P360 34.1%L309 32.4%F359 29.4% | P360 46.5%F356 36.2%M376 31.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **A5** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | Y234 86.5%F356 72.9%I380 63.6%M230 54.8%L309 30.9% | H302 28.3%F359 26.2% | Y234 53.5%F356 51.2%M306 49.9%H302 48.9%V305 48.8%L309 39.0%M230 33.3%F359 28.9% | I380 37.5%H302 31.6% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T6** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | M230 91.5%H226 55.4%H302 35.5%Y234 25.1% | V293 41.7%H302 37.2%S292 34.3%L298 32.6% | H302 73.4%M230 53.4%V305 30.1%Y234 29.0% | M230 27.6%H302 26.5% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **A8** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | H377 99.4%I380 98.6%M376 67.5% | I380 48.7%H377 48.4%M376 29.8%F359 28.0% | I380 90.9%H377 77.3%F356 39.8% | H377 73.2%I380 53.3%M376 36.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T9** | **CTR H bonds** | H226 5.0% | / | H226 6.4% | / |
| **CTR Contacts** | I198 93.8%L202 81.5%H201 56.6%I380 36.7%V206 27.5%H381 25.6% | I380 39.5%I198 36.3%F356 25.2% | I198 89.4%L202 80.8%H201 49.1%H226 46.9% | I198 60.1%L202 51.8%H381 33.1%I380 30.5%H201 30.5% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **Q10** | **CTR H bonds** | / | / | S292 7.1% | W290 5.0% |
| **CTR Contacts** | S292 46.3%H201 30.0%L291 27.3% | S292 38.9%M230 29.7%H226 27.8%V293 25.4% | W290 68.7%S292 44.6%H201 28.1%H226 27.5%L291 26.8% | S292 57.5%W290 57.0%H226 29.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **K11** | **CTR H bonds** | D373 31.9% | D373 31.0% | D373 55.1% | D373 63.8% |
| **CTR Contacts** | R362 91.7%D373 87.3%H377 82.5% | H377 61.8%D373 39.4%R362 35.2% | H377 79.6%D373 63.8%R362 60.9% | D373 63.2%R362 61.6%H377 32.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L12** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | L148 99.8%H377 99.6%Y149 98.9%A153 97.7%L202 92.6%H381 73.4%A145 50.8%V206 25.1% | L202 57.1%Y149 54.9%L148 51.8%A152 50.8%H381 38.9%H377 35.5%H156 34.5%I198 32.9%A145 32.9% | L148 99.6%Y149 96.1%A152 95.9%L202 94.2%H377 86.9%H381 80.9%A145 62.5% | Y149 90.8%L148 85.1%A145 85.1%L202 70.2%H377 61.4%A152 49.8%H381 33.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **A13** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | V205 73.4%L202 45.6%V206 32.5% | V205 64.3%W290 33.9% | V205 69.9%W290 68.8%L202 56.5%V206 29.0% | V205 88.4%W290 77.4%L202 23.3% |
| **RAMP H bonds** | // | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N14** | **CTR H bonds** | / | / | S292 5.7% | / |
| **CTR Contacts** | S292 33.6% | V293 44.8%W290 28.5% | S292 87.0%W290 52.9%V293 36.9% | W290 66.4%S292 25.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **F15** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | A145 99.8%L148 89.2%N144 63.6%K141 57.0%K370 46.7%Y374 27.9% | A145 66.5%L148 52.2%K141 39.0%Y149 32.9% | A145 99.7%K141 75.4%L148 58.8%L142 45.2%N144 40.8% | K141 95.2%A145 94.1%L142 55.7%N144 47.8%L148 46.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L16** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | A145 98.9%Y149 98.5%V206 97.7%Y146 86.3%L142 78.5%V205 60.2%L202 35.3%P207 27.6% | V205 80.9%Y149 65.2%V206 63.7%L202 58.5%A145 57.1%Y146 37.5%N208 36.9%L142 35.7% | Y149 99.2%V206 97.9%A145 94.0%Y146 89.7%L142 67.5%V205 63.6%L202 52.6% | V205 84.0%L142 83.6%A145 79.6%Y149 75.9%Y146 75.7%V206 64.5%N208 46.1%L202 37.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **V17** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | N208 86.5%V205 62.5%W290 29.7% | V205 51.6%N208 50.3%V212 30.6%W290 30.4% | V205 63.1%W290 40.9%G209 36.6%V206 35.0%N208 34.6% | N208 81.8%W290 70.2%V205 57.7%G209 49.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **I26** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | L142 32.1% | / | L142 27.8% | L40 73.2%R213 50.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L27** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | L40 35.8%Y41 28.5% | L142 53.6%L40 42.2%P100 41.0%Y41 39.2%D101 32.2%P139 31.1%T138 29.8% | / | Y41 92.5%L40 73.9%P100 54.2%F99 49.3% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S28** | **CTR H bonds** | D101 5.0% | / | / | D101 12.1% |
| **CTR Contacts** | D101 30.0% | D101 43.1% | Y41 25.8% | D101 41.9% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S29** | **CTR H bonds** | D101 34.6% | D101 36.2% | D101 32.7% | D101 23.9% |
| **CTR Contacts** | D101 97.3%N135 53.2% | D101 97.7%N135 72.0% | D101 74.5%N135 44.1%T138 25.5% | D101 96.3%N135 22.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T30** | **CTR H bonds** | D101 60.8% | D101 56.8% | D101 45.1% | D101 66.36% |
| **CTR Contacts** | D101 89.1%F102 86.9%F99 86.0%W79 64.6%N135 61.0% | D101 95.3%F99 88.7%N135 86.0%F102 85.0%W79 48.9%Y41 36.1% | D101 71.0%N135 67.0%F99 65.3%W79 57.1%F102 56.5% | F99 75.2%W79 62.5%F102 61.2%N135 32.5%Y41 25.2 |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N31** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | W79 51.4% | W79 47.9% | W79 43.5% | W79 53.3% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **V32** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | Y131 94.8%F102 94.8%T132 83.7%W79 76.3%N135 70.9%W128 33.6% | T132 57.1%N135 56.7%F102 54.8%Y131 53.6%W79 43.2% | Y131 71.4%F102 71.0%N135 64.0%T132 63.5%W79 62.2% | F102 89.9%Y131 Y131 84.0%W79 70.6%N135 48.7%T132 47.6% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **G33** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | W128 37.2% | / | W128 28.0% | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S34** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | H121 27.6% | / | W128 35.7%H121 30.4% | / |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N35** | **CTR H bonds** | R126 5.4% | / | / | / |
| **CTR Contacts** | R126 42.5%N124 36.4% | R126 29.6%N124 28.8% | W128 28.3% | N124 36.8%R126 30.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | F83 53.3% | / |  |  |
| **T36** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | W128 62.0%W79 48.8%R126 31.3% | W128 47.0%W79 45.2%Y131 27.8%T132 25.1% | W128 26.7% | W79 84.6%W128 43.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **Y37** | **CTR H bonds** | S129 16.1% (sb) | S129 16.7% (sb) | D77 6.4% | S129 37.5%R45 16.9% |
| **CTR Contacts** | W79 91.6%G78 80.9%Y131 67.7%D77 64.1%S129 52.9%W128 41.4% | Y131 84.7%W79 82.4%S129 56.2W128 54.0%D77 53.0%G78 47.6%R126 41.7%N130 31.2% | W128 33.5% | W79 92.3%G78 82.5%D77 61.3%Y131 55.1%S129 53.7%W128 44.5%M48 35.8%R45 32.7%F99 29.6%Y41 27.2% |
| **RAMP H bonds** | W84 11.9% (sb) | W84 5.9% (sb) |  |  |
| **RAMP Contacts** | W84 85.8%P85 75.2%W74 35.6%F83 32.4% | W84 50.4%F83 50.1%P85 44.8% |  |  |

**Supplementary modelling Table SM4.**

Hydrogen bonds and general contacts established between amylin and CTR in presence (RAMP1) and without RAMP1 (NO RAMP1). Data are from three MD and metadynamics simulation replicas performed on amylin in its free carboxylic (T37-COO-) C terminus form. Percentages refer to the total number of frames of joint MD replicas. (sb): hydrogen bond involving an amylin side chain and the backbone of the CTR (or RAMP1); (bb): hydrogen bond involving the amylin backbone and the backbone of the CTR (or RAMP1); (bs): hydrogen bond involving amylin backbone and a CTR side chain (or RAMP1); if not specified, hydrogen bond refers to both the amylin and the receptor side chains. Only values higher than 5% for hydrogen bonds and 25% for general contacts are reported.

|  |  |
| --- | --- |
| **Amylin****Residue** | **Y37-COO-** |
| **RAMP1** | **NO RAMP1** |
| **Classic MD****Bound state** | **Metadynamics****Bound state** | **Classic MD****Bound state** | **Metadynamics****Bound state** |
| **K1** | **CTR H bonds** | / | / | D97 10.9% | / |
| **CTR Contacts** | W361 64.1%L298 30.2%H302 28.5% | T295 25.8% | T295 46.7%V293 28.9%P104 27.4%D97 26.4% | P360 25.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N3** | **CTR H bonds** | / | T295 11.7% | / | / |
| **CTR Contacts** | H302 90.2%M306 71.2%V305 37.5% | H302 64.5%L298 56.6%T295 51.6% | H302 86.0%L298 76.0%T295 33.0% | Y299 52.9%L298 48.8%M306 48.6%H302 45.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T4** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | F356 77.2%P360 58.6%M376 47.1%M306 38.3%L309 34.3%Y372 30.2%Y234 28.1%V305 26.6%I380 25.4% | H302 62.5%P360 51.7%W361 42.9%M306 41.3% | H302 78.6%M306 76.9%P360 47.9%F356 43.2% | P360 43.8%M306 37.0%F356 34.2%L309 30.8%W361 30.7%V357 29.8%F359 25.7%R362 25.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **A5** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | Y234 92.0%M230 74.5%I380 47.1%F356 45.8%H302 29.5% | H302 37.1%Y234 32.1%F356 26.8% | H302 80.6%Y234 52.7%V305 45.8%M306 44.2%L309 40.7%M230 29.4% | N208 85.4%H302 43.3%Y234 41.1%V305 38.4%M306 27.4%M230 26.7%L309 25.9% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T6** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | M230 55.6%H302 54.4%Q227 41.9%H226 35.7%Y234 31.7%V305 26.3% | L291 30.8%M230 28.9%Y234 25.1% | H302 48.9%M230 43.8% | H302 43.2%L298 27.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **A8** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | I380 99.1%H377 92.7%M376 39.8%F356 28.8% | H377 57.1%I380 53.1%M376 43.1% | I380 97.5%H377 69.1%F356 40.4% | I380 64.0%H377 44.0%F356 28.8% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T9** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | I198 99.6%L202 82.8%H201 76.2%H226 52.4%M230 29.6% | I198 61.8%L202 58.4%H201 57.8%H226 29.3%I380 28.6% | I198 85.9%L202 52.7%H226 38.7%H381 35.5%H201 30.5%I380 30.0% | I198 74.8%H226 54.3%L202 49.5%H201 41.3%M230 35.3%I380 32.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **Q10** | **CTR H bonds** | / | / | / | H226 5.0% |
| **CTR Contacts** | W290 74.1%H201 61.1%H226 48.3%H223 28.6% | W290 56.4%H201 45.6%S292 33.4%H226 31.5% | H226 52%W290 49.4%H201 34.1%Q227 26.0% | H226 61.0%M230 44.3%Q227 44.2%H201 33.4%H223 26.9% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **K11** | **CTR H bonds** | D373 89.0% | D373 70.7% | D373 60.3%H377 14.1% | D373 61.1%H377 8.1% |
| **CTR Contacts** | R362 99.5%D373 99.0%H377 82.8%K370 33.0% | R362 84.7%D373 83.8%H377 63.8% | D373 82.8%H377 76.3%R362 73.9% | D373 74.1%R362 70.4%H377 58.7% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L12** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | H377 99.5%L148 99.4%L202 98.1%A152 97.8%Y149 95.7%H381 85.1%A145 28.3% | Y149 91.6%L148 88.9%L202 79.4%A145 71.6%A152 60.3%H377 57.8%H381 39.7% | L148 99.3%Y149 98.5%A145 95.8%H377 94.8%A152 85.6%L202 76.1%H381 63.7% | L148 94.5%L202 88.0%Y149 83.9%H377 78.6%A145 73.8%A152 63.9%H381 53.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **A13** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | V205 99.9%L202 48.2%W290 41.0%H201 40.3% | V205 89.4%H201 38.9%L202 36.4% | V205 95.1%L202 51.2%H201 48.7%W290 36.7% | V205 79.5%L202 75.7%H201 62.3%W290 35.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N14** | **CTR H bonds** | / | / | W290 6.1% | / |
| **CTR Contacts** | / | / | S292 57.8%W290 40.2% | W290 34.1% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **F15** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | A145 99.4%L148 76.9%K370 44.8%N144 41.7%Y374 33.9%L142 28.7%D373 28.3%H377 26.6% | A145 98.3%L142 67.1%K141 48.3%L148 40.4%H377 31.2% | A145 97.1%K141 75.4%L142 72.8%L148 29.4% | A145 91.6%K141 73.8%L142 53.8%L148 46.6%N144 25.3% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | Y120 28.0% | / |  |  |
| **L16** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | Y149 99.3%V205 99.2%V206 93.9/A145 89.9%Y146 88.9%L202 65.1%N208 55.4%L142 50.8% | V205 85.2%Y149 85.2%A145 84.5%Y146 73.5%L142 73.3%V206 69.2%L202 43.4%N208 32.1% | Y149 99.2%V205 96.6%Y146 86.5%A145 84.5%V206 65.9%L142 64.3%L202 55.4%N208 50.2% | V205 96.1%Y149 90.4%V206 88.0%A145 84.1%Y146 82.4%L202 73.8%L142 49.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **V17** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | V205 93.1%N208 82.7%G209 81.8%W290 41.8% | V205 63.2%N208 52.3%G209 29.4%W290 27.5% | V205 97.1%N208 77.5%V212 44.4%G209 42.5% | V205 92.3%W290 87.8%G209 80.1%N208 45.5% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **I26** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | N208 65.3%L142 61.6%T138 49.8%D101 39.5%P139 33.1% | N208 42.5%L40 36.6% | / | N208 57.0%E210 30.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **L27** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | N208 39.0% | T41 76.1%P100 70.2%L40 63.1% | L40 32.6%Y41 31.8% | P100 72.6%Y41 57.0%L40 52.1%D101 39.0%L142 38.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S28** | **CTR H bonds** | / | / | D101 5.4% | / |
| **CTR Contacts** | P100 29.1%D101 26.7% | Y41 37.5% | Y41 24.3% | Y41 68.7% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S29** | **CTR H bonds** | D101 42.3% | D101 43.2% | D101 30.0% | D101 16.3% |
| **CTR Contacts** | D101 82.4%N135 65.6% | D101 99.3%N135 83.4% | D101 68.9%Y41 36.4%N135 33.4% | D101 99.0%N135 67.3% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T30** | **CTR H bonds** | D101 47.9% | D101 58.6% D | D101 38.4% | D101 69.4% |
| **CTR Contacts** | F99 73.9%N135 69.3%F102 63.6%D101 63.1%P100 50.3%W79 25.9% | F102 98.3%D101 94.9%N135 93.2%F99 92.0%W79 85.4% | F99 81.1%Y41 58.1%D101 57.6%N135 47.5%F102 47.1%W79 39.1% | N135 100.0%D101 100.0%F99 99.8%F102 99.5%W79 84.6%Y41 32.4% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N31** | **CTR H bonds** | / | / | / |  |
| **CTR Contacts** | W79 23.6% | W79 88.9% | W79 50.0%Y41 26.0% |  |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **V32** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | Y131 74.6%F102 65.4%T132 52.9%W79 44.9%N135 40.4% | Y131 99.5%T132 99.3%F102 98.6%N135 94.6%W79 83.3%W128 52.1% | W79 74.1%Y131 54.2%F102 50.9%T132 44.7%N135 44.1%Y41 32.6%M48 30.6%F99 30.2%G44 28.9%Y98 27.6% | W79 96.7% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **G33** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | / | W128 61.5% | W79 33.3%M48 29.9% | W128 61.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **S34** | **CTR H bonds** | / | N124 24.4% | / | N124 24.5% |
| **CTR Contacts** | W128 25.4% | N124 89.4%H121 84.3%W128 71.3%R126 63.4% | G78 25.3% | N124 90.6%W128 80.8%R126 76.2%H121 76.0% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **N35** | **CTR H bonds** | / | N124 5.9% | / | R126 7.9% |
| **CTR Contacts** | R126 37.8% | N124 86.8%R126 70.6% | G78 25.4% | R126 83.6%N124 58.9% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **T36** | **CTR H bonds** | / | / | / | / |
| **CTR Contacts** | W79 27.9% | W79 67.2% | / | W79 96.2% |
| **RAMP H bonds** | / | / |  |  |
| **RAMP Contacts** | / | / |  |  |
| **Y37** | **CTR H bonds** | R45 28.6%R126 8.2% | R126 11.5% | R126 28.2%R45 13.0% | R45 95.6%W79 21.6% |
| **CTR Contacts** | W79 45.3%R126 37.3%R45 30.5%G78 25.0% | W79 98.0%G78 84.8%D77 48.6% | R126 45.8% | R45 81.7%W79 69.3%M48 41.4%G78 29.8% |
| **RAMP H bonds** | / | W74 13.9% |  |  |
| **RAMP Contacts** | W84 25.9% | W84 98.0%P85 81.7%W74 65.2% |  |  |

**Supplementary modelling Table SM5.**

Hydrogen bonds established by CTR residues located at the ECL2, during simulation with amylin (mutant Q10A) bound. (sb): hydrogen bond involving an amylin side chain and the backbone of the CTR (or RAMP1); (bb): hydrogen bond involving the amylin backbone and the backbone of the CTR (or RAMP1); (bs): hydrogen bond involving amylin backbone and a CTR side chain (or RAMP1); if not specified, hydrogen bond refers to both the amylin and the receptor side chains. Only values higher than 5% are reported.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **CTR EL2 Residue** | **Hydrogen bond counterpart** | **Bound state (RAMP1)** | **Bound state (NO RAMP1)** | **Q10A****Bound state (RAMP1)** |
| **W290** | **Amy** | / | / | / |
| **CTR** | / | / | D287 55.4% (bs) |
| **L291** | **Amy** | / | / | / |
| **CTR** | / | D287 18.1% (bs) | D287 14.9% |
| **S292** | **Amy** | / | N14 5.7% | / |
| **CTR** | / | / | D287 49.8% (bs)D287 70.8% |
| **V293** | **Amy** | / | / | / |
| **CTR** | / | / | / |
| **E294** | **Amy** | K1 8.8% (bb)K1 4.2% (sb) | K1 3.3% (bb) | K1 14.8% (sb) |
| **CTR** | R281 34.3%H296 7.6% (sb)T295 3.2% (sb) | R281 40.0%H296 24.7% (sb)T295 12.9% (sb) | H296 17.8% (sb)T295 17.2% (sb)R281 9.0% |
| **T295** | **Amy** | / | / | / |
| **CTR** | E294 3.2% (bs) | E294 12.9% (bs) | E294 17.2% (bs)N286 7.0% |
| **H296** | **Amy** | / | / | / |
| **CTR** | E294 7.6% (bs) | H296 24.7% (bs)R281 5.8% (bs) | E294 17.8% (bs)R281 12.4% (bs) |