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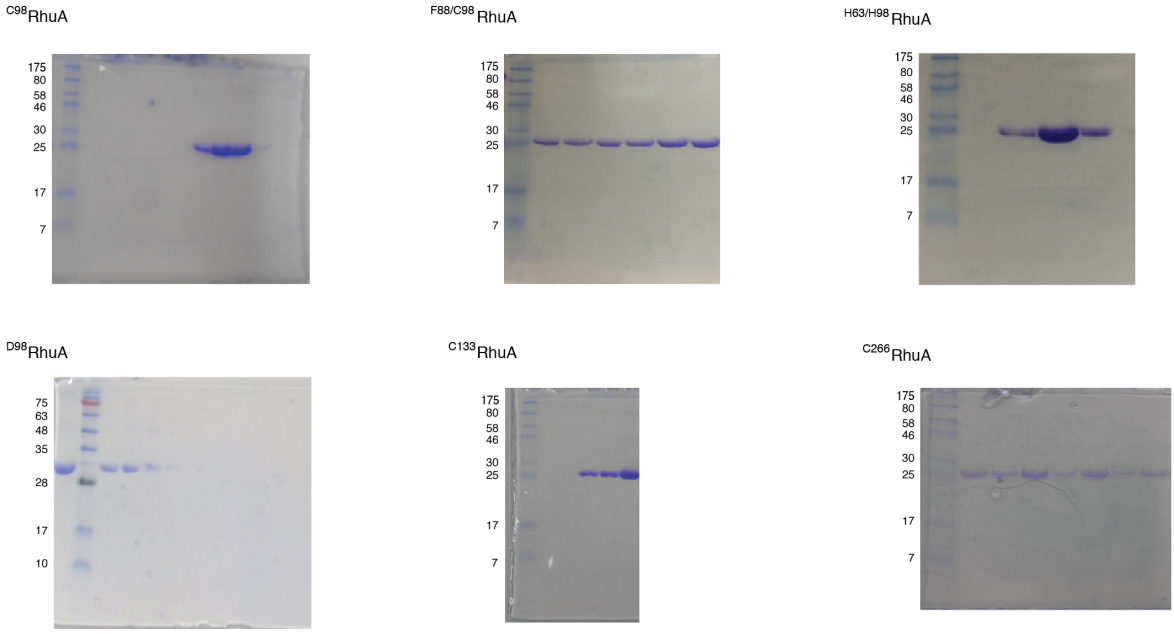
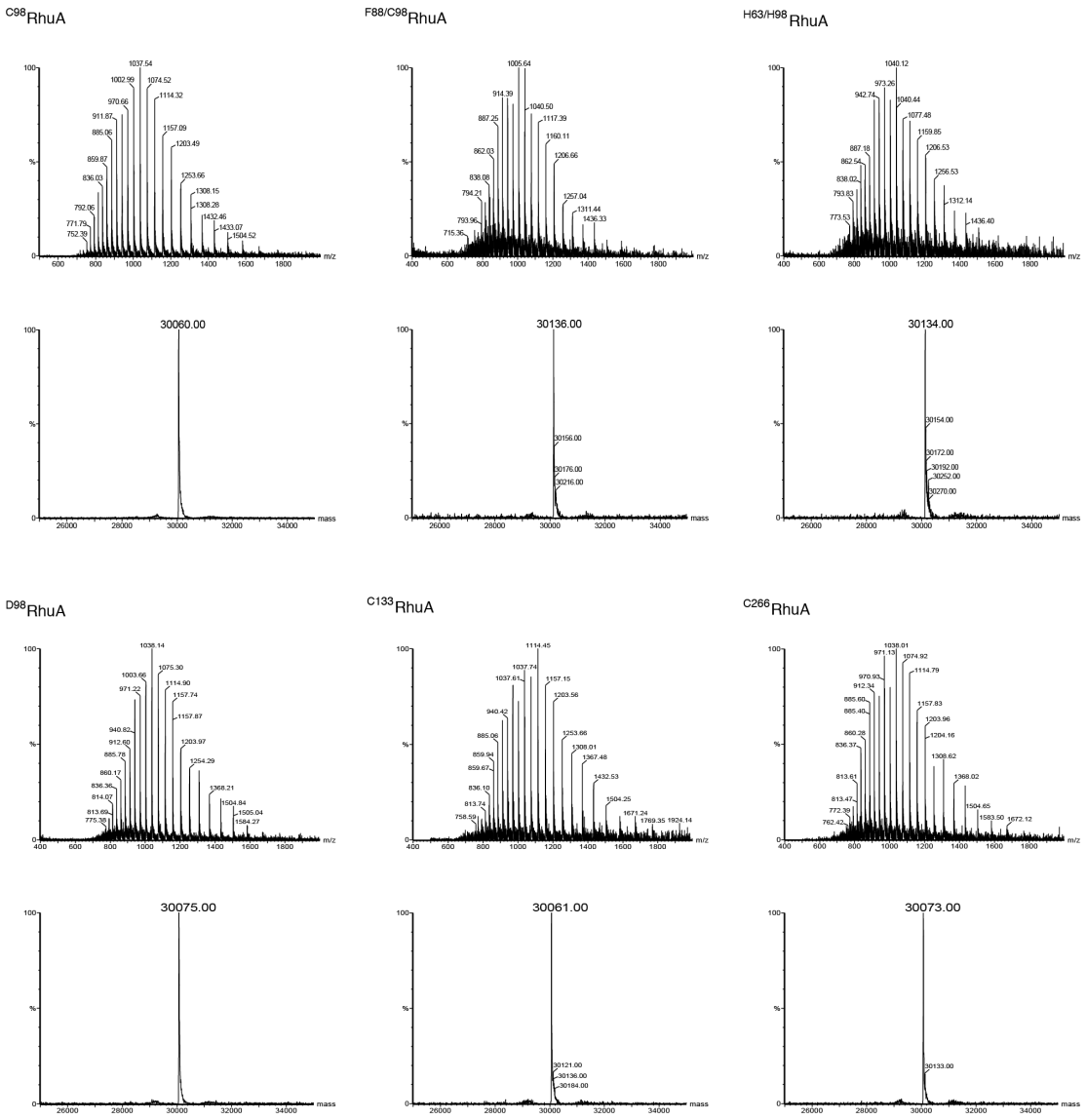
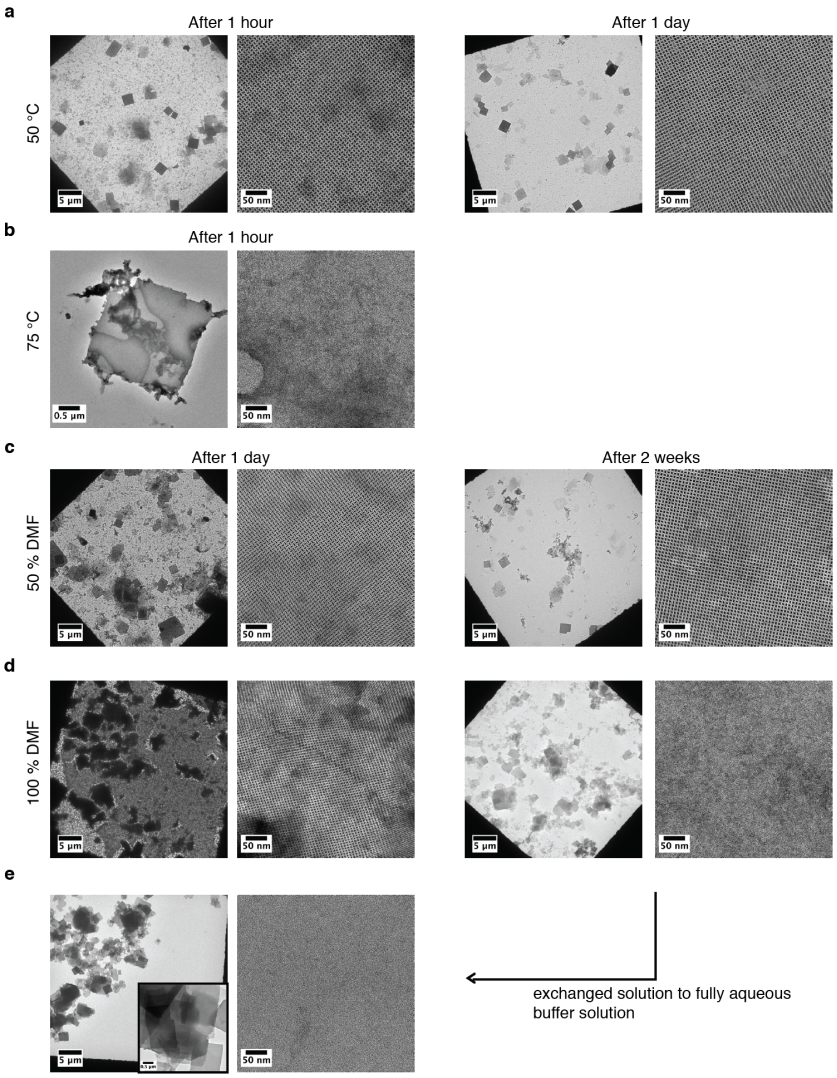



**Supplementary Figure 1 | SDS-PAGE characterization of RhuA mutants.**




**Supplementary Figure 2 | Positive ESI-MS characterization of RhuA mutants**. Top spectra display the raw data showing the cluster ions. The bottom panels are the deconvoluted spectra.




**Supplementary Figure 3 | Thermal and organic-solvent stability of $^{C98}$RhuA crystals**. **a-d**, $^{C98}$RhuA crystals were incubated at 50 °C, 75 °C, 50% DMF/$H_2O$ and 100% DMF for indicated periods and characterized by TEM. The lattices were found to be stable at 50 °C for prolonged periods and retained their crystallinity, whereas at 75 °C, both the lattice morphology and the crystallinity were lost due likely to the denaturation of $^{C98}$RhuA building blocks. Likewise, in 50 50% DMF/$H_2O$ solutions, the lattices were indefinitely stable and crystalline, but appear to gradually lose both properties in 100% DMF. Interestingly, the lattice morphologies were retained, suggesting that the $^{C98}$RhuA building blocks likely denatured but still remained tightly associated with one another within the framework. **e**, The crystallinity of the samples in 100% DMF could not be recovered upon exchange into aqueous buffer, indicating that protein denaturation is irreversible.

**Supplementary Table 1 | List of primers used in constructing site-directed mutants of RhuA using $^{C98}$RhuA as a base.**

| Variant | Mutation | Primer Sequence |
|---|---|---|
| $^{F88/C98}$RhuA | A88F | 5'-CGCAATGTCCAGTTGGACCCAGCGTTTAACCTGGGCATTGTTAAGGTGG-3'<br>5'-CCACCTTAACAATGCCCAGGTTAAACGCTGGGTCCAACTGGACATTGCG-3' |
| $^{H63/H98}$RhuA | P63H | 5'- CGCTGAGCCAGCCGATGCATCTGTTGGCGAATACCC -3'<br>5'- GGGTATTCGCCAACAGATGCATCGGCTGGCTCAGCG -3' |
| | C98H | 5'- GGGCATTGTTAAGGTGGATAGCCATGGTGCAGGTTACCACATCC-3'<br>5'- GGATGTGGTAACCTGCACCATGGCTATCCACCTTAACAATGCCC -3' |
| $^{D98}$RhuA | C98D | 5'- GCATTGTTAAGGTGGATAGCGACGGTGCAGGTTACCACATCC -3'<br>5'- GGATGTGGTAACCTGCACCGTCGCTATCCACCTTAACAATGC -3' |
| $^{C133}$RhuA | N133C | 5'- GCGAGCGTATCAAGGCGACCTGCGGCAAAGACCGCG-3'<br>5'- CGCGGTCTTTGCCGCAGGTCGCCTTGATACGCTCGC-3' |
| $^{C266}$RhuA | T266C | 5'- GGTAAGCGTTTTGGTGTCTGTCCGCTGGCGTCCGCGCTGG-3'<br>5'- CCAGCGCGGACGCCAGCGGACAGACACCAAAACGCTTACC-3' |

**Supplementary Table 2 | Amino acid sequences of RhuA variants.** Point mutation positions are underlined.

| Variant | Amino Acid Sequence |
|---|---|
| $^{C98}$RhuA | MQNITQSWFVQGMIKATTDAWLKGWDERNGGNLTLRLDDADIAPYHDNFHQQPRYIPLS QPMPLLANTPFIVTGSGKFFRNVQLDPAANLGIVKVDS**C**GAGYHILWGLTNEAVPTSELPA HFLSHSERIKATNGKDRVIMHCHATNLIALTYVLENDTAVFTRQLWEGSTECLVVFPDGVG ILPWMVPGTDAIGQATAQEMQKHSLVLWPFHGVFGSGPTLDETFGLIDTAEKSAQVLVKV YSMGGMKQTISREELIALGKRFGVTPLASALAL |
| $^{H63/H98}$RhuA | MQNITQSWFVQGMIKATTDAWLKGWDERNGGNLTLRLDDADIAPYHDNFHQQPRYIPLS QPM**H**LLANTPFIVTGSGKFFRNVQLDPAANLGIVKVDS**H**GAGYHILWGLTNEAVPTSELPA HFLSHSERIKATNGKDRVIMHCHATNLIALTYVLENDTAVFTRQLWEGSTECLVVFPDGVG ILPWMVPGTDAIGQATAQEMQKHSLVLWPFHGVFGSGPTLDETFGLIDTAEKSAQVLVKV YSMGGMKQTISREELIALGKRFGVTPLASALAL |
| $^{F88/C98}$RhuA | MQNITQSWFVQGMIKATTDAWLKGWDERNGGNLTLRLDDADIAPYHDNFHQQPRYIPLS QPMPLLANTPFIVTGSGKFFRNVQLDPA**F**NLGIVKVDS**C**GAGYHILWGLTNEAVPTSELPA HFLSHSERIKATNGKDRVIMHCHATNLIALTYVLENDTAVFTRQLWEGSTECLVVFPDGVG ILPWMVPGTDAIGQATAQEMQKHSLVLWPFHGVFGSGPTLDETFGLIDTAEKSAQVLVKV YSMGGMKQTISREELIALGKRFGVTPLASALAL |
| $^{D98}$RhuA | MQNITQSWFVQGMIKATTDAWLKGWDERNGGNLTLRLDDADIAPYHDNFHQQPRYIPLS QPMPLLANTPFIVTGSGKFFRNVQLDPAANLGIVKVDS**D**GAGYHILWGLTNEAVPTSELPA HFLSHSERIKATNGKDRVIMHCHATNLIALTYVLENDTAVFTRQLWEGSTECLVVFPDGVG ILPWMVPGTDAIGQATAQEMQKHSLVLWPFHGVFGSGPTLDETFGLIDTAEKSAQVLVKV YSMGGMKQTISREELIALGKRFGVTPLASALAL |
| $^{C133}$RhuA | MQNITQSWFVQGMIKATTDAWLKGWDERNGGNLTLRLDDADIAPYHDNFHQQPRYIPLS QPMPLLANTPFIVTGSGKFFRNVQLDPAANLGIVKVDSDGAGYHILWGLTNEAVPTSELPA HFLSHSERIKAT**C**GKDRVIMHCHATNLIALTYVLENDTAVFTRQLWEGSTECLVVFPDGVG ILPWMVPGTDAIGQATAQEMQKHSLVLWPFHGVFGSGPTLDETFGLIDTAEKSAQVLVKV YSMGGMKQTISREELIALGKRFGVTPLASALAL |
| $^{C266}$RhuA | MQNITQSWFVQGMIKATTDAWLKGWDERNGGNLTLRLDDADIAPYHDNFHQQPRYIPLS QPMPLLANTPFIVTGSGKFFRNVQLDPAANLGIVKVDSDGAGYHILWGLTNEAVPTSELPA HFLSHSERIKATNGKDRVIMHCHATNLIALTYVLENDTAVFTRQLWEGSTECLVVFPDGVG ILPWMVPGTDAIGQATAQEMQKHSLVLWPFHGVFGSGPTLDETFGLIDTAEKSAQVLVKV YSMGGMKQTISREELIALGKRFGV**C**PLASALAL |

**Supplementary Table 3 | Molecular masses of RhuA mutants.** For the actual mass spectra, refer to Supplementary Figure 2.

| Variant | Calculated Mass (Da) | Observed Mass (Da) |
|---|---|---|
| $^{C98}$RhuA | 30059.5 | 30060.0 |
| $^{F88/C98}$RhuA | 30135.6 | 30136.0 |
| $^{H63/H98}$RhuA | 30133.5 | 30134.0 |
| $^{D98}$RhuA | 30071.4 | 30075.0 |
| $^{C133}$RhuA | 30060.4 | 30061.0 |
| $^{C266}$RhuA | 30073.4 | 30073.0 |

**Supplementary Table 4 | Symmetry analysis for the 2D lattices of RhuA variants.** Internal phase residuals for all possible non-hexagonal plane groups. Residuals were determined from the power spectra shown in Extended Data Fig. 10a using the program ALLSPACE, as described in Methods. Reflections up to 11 Å and with IQ less than 6 were included in the calculations.

**a,** $^{C98}$RhuA

| 2D plane group | Phase Residual[a] (°) | Number of comparisons | Target Residual[b] (°) |
|---|---|---|---|
| p1 | 22.4[c] | 126 | |
| p2 | 29.6* | 63 | 32.5 |
| p12_b | 74.6 | 45 | 23.1 |
| p12_a | 73.8 | 45 | 23.1 |
| $p12_1$_b | 18.1* | 45 | 23.1 |
| $p12_1$_a | 12.8* | 45 | 23.1 |
| c12_b | 74.6 | 45 | 23.1 |
| c12_a | 73.8 | 45 | 23.1 |
| p222 | 56.1 | 153 | 26.6 |
| $p222_1$_b | 54.2 | 153 | 26.6 |
| $p222_1$_a | 56.6 | 153 | 26.6 |
| $p22_12_1$ | 21.6* | 153 | 26.6 |
| c222 | 56.1 | 153 | 26.6 |
| p4 | 26.7* | 155 | 26.5 |
| p422 | 52.6 | 333 | 24.3 |
| $p42_12$ | 24.4* | 333 | 24.3 |

[a] Phase residual versus other spots (90° = random).
[b] Expected residual based on the quality of the reflections involved in the calculation.
[c] Theoretical, based on the quality of the reflections observed

* acceptable (better than target or within 1°)
† to be considered (within 5° from target)
§ possibility (within 10° from target)

**b,** $^{H63/H98}$RhuA

| 2D plane group | Phase Residual[a] (°) | Number of comparisons | Target Residual[b] (°) |
|---|---|---|---|
| p1 | 21.8[c] | 96 | |
| p2 | 29.4[*] | 48 | 31.6 |
| p12_b | 44.7 | 28 | 23.2 |
| p12_a | 46.0 | 31 | 24.0 |
| $p12_1$_b | 73.0 | 28 | 23.2 |
| $p12_1$_a | 79.5 | 31 | 24.0 |
| c12_b | 44.7 | 28 | 23.2 |
| c12_a | 46.0 | 31 | 24.0 |
| p222 | 38.5 | 107 | 26.2 |
| $p222_1$_b | 64.7 | 107 | 26.2 |
| $p222_1$_a | 70.9 | 107 | 26.2 |
| $p22_12_1$ | 62.6 | 107 | 26.2 |
| c222 | 38.5 | 107 | 26.2 |
| p4 | 23.1[*] | 112 | 26.0 |
| p422 | 31.9[§] | 231 | 23.8 |
| $p42_12$ | 62.1 | 231 | 23.8 |

[a] Phase residual versus other spots (90° = random).
[b] Expected residual based on the quality of the reflections involved in the calculation.
[c] Theoretical, based on the quality of the reflections observed

[*] acceptable (better than target or within 1°)
[†] to be considered (within 5° from target)
[§] possibility (within 10° from target)

**c,** $^{F88/C98}$RhuA

| 2D plane group | Phase Residual[a] (°) | Number of comparisons | Target Residual[b] (°) |
|---|---|---|---|
| p1 | 26.8[c] | 160 | |
| p2 | 26.4* | 80 | 39.3 |
| p12_b | 71.2 | 54 | 27.7 |
| p12_a | 70.4 | 56 | 28.1 |
| $p12_1$_b | 14.1* | 54 | 27.7 |
| $p12_1$_a | 13.1* | 56 | 28.1 |
| c12_b | 71.2 | 54 | 27.7 |
| c12_a | 70.4 | 56 | 28.1 |
| p222 | 52.1 | 190 | 32.1 |
| $p222_1$_b | 56.9 | 190 | 32.1 |
| $p222_1$_a | 58.3 | 190 | 32.1 |
| $p22_12_1$ | 19.3* | 190 | 32.1 |
| c222 | 52.1 | 190 | 32.1 |
| p4 | 17.3* | 204 | 31.7 |
| p422 | 45.7 | 431 | 29.1 |
| $p42_12$ | 16.0* | 431 | 29.1 |

[a] Phase residual versus other spots (90° = random).
[b] Expected residual based on the quality of the reflections involved in the calculation.
[c] Theoretical, based on the quality of the reflections observed

* acceptable (better than target or within 1°)
† to be considered (within 5° from target)
§ possibility (within 10° from target)