Supplementary Materials

Analysis of procollagen C-proteinase enhancer-1/glycosaminoglycan binding sites and of the potential role of calcium ions in the interaction

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| Table S1. Molecular docking MD-based analysis summary for CUB1-CUB2 domain/GAG interaction. |
|-----------------------------------------------|
| GAG  | 1m, ε | # | Size | ΔG, kcal/mol | Top_{MM-GBSA} 10 residues for GAG binding | Polarity |
|------|--------|---|------|--------------|------------------------------------------|----------|
| CS6, dp4 | 3, 3   | 1 | 17   | -20.2±12.1   | A64, R66, R85, Y187, R189, R206, K209, F210, C211, P216 | 16/1 |
|      |        | 2 | 9    | -24.1±6.7, -50.6±5.4 | A64, R66, R82, L83, G84, R85, Y187, R189, R206, V231 | 8/1 |
|      |        | 3 | 5    | -4.8±6.0, -27.9±6.7 | R82, G84, R85, P92, A93, Y187, R189, S204, R206, K209 | 5/0 |
|      |        | 4 | 5    | -22.2±7.2, -30.7±11.5 | A64, R66, R82, R85, F86, F90, Y187, R189, R206, K209 | 4/1 |
| CS6, dp6 | 3, 3   | 1 | 17   | -28.8±7.8, -21.6±6.5, -13.6±8.4, -31.0±11.0 | H62, R66, R82, R85, Y187, C188, R189, R206, K209, F210 | 17/0 |
|      |        | 2 | 3    | -34.9±8.2, -25.7±9.6 | A64, R66, R82, R85, R105, C188, R189, R206, K209 | 2/1 |
|      |        | 2 | 3    | -34.9±8.2, -25.7±9.6 | A64, R66, R82, R85, R105, T186, C188, R189, R206, K209 | 2/1 |
|      |        | 3 | 3    | -6.3±13.1, -7.4±15.4 | R66, R82, R85, F86, C87, F180, L180, A199, D202 | 3/0 |
Table S2. Similarity of GAG binding poses for the CUB1-CUB2 domains as of the number of common amino acid residues identified in the top 10 for binding according to MM-GBSA calculations per cluster.

| GAG   | CS6, dp4 | CS6, dp6 | DS, dp6 | HP, dp2 | HP, dp4 | HP, dp6 |
|-------|----------|----------|---------|---------|---------|---------|
| CS6, dp4 | 10 6 5 7 7 5 6 6 7 7 7 5 6 7 6 7 | 6 10 6 7 6 5 6 7 8 7 8 6 6 6 6 7 | 5 6 10 6 6 5 5 7 7 6 7 6 6 6 6 6 |
|       | 7 7 6 10 7 6 7 7 8 9 8 6 7 7 7 8 | 6 6 6 7 7 10 7 8 7 7 7 7 7 7 7 7 |
| CS6, dp6 | 7 6 6 7 10 6 7 7 7 7 7 6 6 7 8 7 7 | 6 6 6 7 7 10 7 8 7 7 7 7 7 7 7 7 |
| DS, dp6 | 5 5 5 6 6 10 7 7 7 6 7 8 9 8 8 8 | 6 6 5 7 7 7 10 7 8 7 7 7 7 7 7 7 |
| HP, dp2 | 6 7 7 7 7 7 7 7 10 9 7 8 9 8 9 8 | 7 8 7 8 7 7 8 9 10 8 9 8 8 8 8 9 |
|       | 7 7 6 9 7 6 7 7 8 10 8 6 7 7 7 8 | 6 6 6 7 7 9 7 8 8 7 8 8 10 9 9 9 |
| HP, dp4 | 7 8 7 8 7 7 7 7 8 9 8 10 8 8 7 8 9 | 5 6 6 6 6 8 7 9 8 6 8 10 8 7 9 8 |
|       | 6 6 6 7 7 9 7 8 8 8 7 8 8 10 9 9 9 | 6 6 6 7 7 8 7 9 8 7 8 8 10 9 9 9 |
| HP, dp6 | 7 6 6 7 8 8 7 8 8 7 7 7 9 10 8 8 | 6 6 6 7 7 8 7 9 8 7 8 8 9 8 9 9 10 |
|       | 7 7 6 8 7 8 8 8 9 8 9 8 9 9 8 9 10 |

Each line/column in front/below each GAG reflects a separate cluster, for which average values were taken into account.

1 DBSCAN parameters \( m \), the minimal neighborhood size and \( \epsilon \), neighborhood search radius \([33]\); 2 Cluster number; 3 Cluster size; 4 Free energy of binding obtained by MM-GBSA; 5 Residues identified in the top 10 for binding according to MM-GBSA calculations per cluster. 6 The polarity of a GAG binding pose was defined as its preferred orientation in relation to the reducing and non-reducing end.
**Table S3.** Similarity of GAG binding poses for the CUB1-CUB2 domains as of the number of common amino acid residues identified in the top 10 for binding according to MM-GBSA calculations per GAG.

| GAG     | CS6, dp4 | CS6, dp6 | DS, dp6 | HP, dp2 | HP, dp4 | HP, dp6 |
|---------|----------|----------|---------|---------|---------|---------|
| CS6, dp4| 10       | 7        | 7       | 9       | 9       | 7       |
| CS6, dp6| 7        | 10       | 7       | 7       | 7       | 7       |
| DS, dp6 | 7        | 7        | 10      | 6       | 8       | 7       |
| HP, dp2 | 9        | 7        | 6       | 10      | 8       | 8       |
| HP, dp4 | 9        | 7        | 8       | 8       | 10      | 8       |
| HP, dp6 | 7        | 7        | 8       | 8       | 10      | 10      |

**Table S4.** Molecular docking MD-based analysis summary for PCPE-1 UNRES models/GAG interaction.

| Structure   | 1\text{m,ε} | 2#   | 3\text{Size} | 4\text{ΔG, kcal/mol} | 5\text{Top}_{\text{MM-GBSA}} 10 residues for GAG binding | 6\text{Polarity} |
|-------------|-------------|------|--------------|-----------------------|----------------------------------------------------------|-----------------|
| Model 1     | 1           | 7    |              | \begin{align*} -90.3 \pm 13.7 \\ -80.2 \pm 16.9 \\ -80.8 \pm 23.0 \end{align*} | R275, R323, K287, R288, R324, K320, K271, L273, K204, K393 | 7/0             |
| HP, dp6     | 2, 2.5      | 2 4  |              | \begin{align*} -87.3 \pm 20.3 \\ -70.1 \pm 13.0 \\ -78.8 \pm 10.6 \end{align*} | R323, R288, R275, K287, K279, K320, R324, K271, L273, G276 | 4/0             |
|             | 3 3         |      |              | \begin{align*} -100.7 \pm 17.4 \\ -86.7 \pm 20.5 \\ -102.2 \pm 21.3 \end{align*} | R323, R288, K287, R324, R275, K320, K279, R422, K393, K271 | 3/0             |
| Model 2     | 1           | 12   |              | \begin{align*} -58.1 \pm 12.6 \\ -59.3 \pm 15.3 \\ -82.5 \pm 23.4 \end{align*} | K394, R348, K393, K295, K293, R324, R422, K299, K305, P298 | 11/1            |
| HP, dp6     | 2, 3.0      | 2 4  |              | \begin{align*} -54.9 \pm 12.9 \\ -10.1 \pm 8.7 \\ -60.0 \pm 14.3 \end{align*} | R288, K295, K293, K344, V294, K287, G289, K394, K204, K271 | 3/1             |
|             | 3 3         |      |              | \begin{align*} -66.7 \pm 15.2 \\ -28.9 \pm 8.7 \\ -46.1 \pm 12.7 \end{align*} | K295, R288, K293, R348, V294, K394, K344, K287, K393, K299 | 3/0             |
| Model 3     | 1           | 14   |              | \begin{align*} -63.1 \pm 11.6 \\ -51.2 \pm 12.6 \\ -67.6 \pm 11.1 \end{align*} | R422, K393, R324, K394, K344, H421, R348, N424, V396, R323, | 12/2            |
| HP, dp6     | 2, 3.2      | 2 8  |              | \begin{align*} -84.0 \pm 10.9 \\ -73.6 \pm 11.4 \\ -90.9 \pm 13.2 \end{align*} | K393, K394, R348, R422, M346, K344, H421, R324, G395, S345 | 7/1             |
|             | 3 3         |      |              | \begin{align*} -61.5 \pm 23.1 \\ -58.3 \pm 9.2 \\ -94.1 \pm 15.6 \end{align*} | K393, K394, R348, K344, R422, G395, K387, K320, V396, Q388 | 2/1             |
Table S5. Molecular docking MD-based analysis summary for NTR domain/Ca\(^{2+}\)/GAG system.

| GAG   | \(m, \varepsilon\) | \(|\bar{\varepsilon}|\) | Size | \(\Delta G\) kcal/mol | Top\_MM-GBSA 10 residues for GAG binding | Polarity |
|-------|---------------------|-----------------|------|---------------------|------------------------------------------|----------|
| HP, dp2 | 4, 0.85             | 1               | 19   | -46.2 ± 7.6         | R323, C389, Q321, C322, P390,             | 19/0     |
|       |                     |                 |      | -37.5 ± 8.0         | P391, R324, P414, Q388, K320             |          |
|       |                     |                 |      | -39.0 ± 12.8        |                                          |          |
|       | 2                   | 10              |      | -42.0 ± 8.7         | R323, R348, K393, R324, K394,            | 10/0     |
|       |                     |                 |      | -20.6 ± 5.9         | K387, K320, Q388, Q321, M346             |          |
|       |                     |                 |      | -33.2 ± 9.6         |                                          |          |
|       | 3                   | 6               |      | -19.6 ± 5.5         | R324, R323, R422, K393, Q321,            | 6/0      |
|       |                     |                 |      | -12.7 ± 9.7         | L420, V419, P391, K320, K387             |          |
|       |                     |                 |      | -14.8 ± 5.8         |                                          |          |
| HP, dp4 | 2, 2.5              | 1               | 17   | -46.7 ± 7.8         | R324, R323, R422, R393, Q321,            | 14/3     |
|       |                     |                 |      | -45.1 ± 10.6        | L420, V419, P391, K320, K387             |          |
|       |                     |                 |      | -52.1 ± 11.4        |                                          |          |
|       | 2                   | 6               |      | -44.9 ± 14.3        | R324, R323, Q321, K393, R422,            | 4/2      |
|       |                     |                 |      | -49.5 ± 7.4         | K320, V419, P391, L420, C322             |          |
|       |                     |                 |      | -39.1 ± 7.9         |                                          |          |
|       | 3                   | 3               |      | -45.5 ± 7.6         | R323, K320, Q321, R422, V419,            | 3/0      |
|       |                     |                 |      | -61.1 ± 8.2         | R322, T327, R324, T325, K393             |          |
|       |                     |                 |      | -62.1 ± 6.7         |                                          |          |
| HP, dp6 | 2, 2.5              | 1               | 3    | -46.9 ± 11.6        | R348, K393, K394, K387, R324,            | 3/0      |
|       |                     |                 |      | -68.0 ± 18.3        | R320, R323, Q388, P391, V347             |          |
|       |                     |                 |      | -50.6 ± 12.7        |                                          |          |
|       | 2                   | 3               |      | -72.8 ± 9.0         | K393, R323, K394, R324, R348,            | 2/1      |
|       |                     |                 |      | -88.6 ± 12.4        | K387, R422, K320, P391, L420             |          |
|       |                     |                 |      | -44.5 ± 6.6         |                                          |          |
|       | 3                   | 3               |      | -42.2 ± 11.2        | R436, K365, R435, G368, K434,            | 3/0      |
|       |                     |                 |      | -31.5 ±11.3         | G367, L369, S336, C437, T366             |          |
|       |                     |                 |      | -18.8 ± 10.6        |                                          |          |

1 DBSCAN parameters \(m\), the minimal neighborhood size and \(\varepsilon\), neighborhood search radius [33]; 2 Cluster number; 3 Cluster size; 4 Free energy of binding obtained by MM-GBSA; 5 Residues identified in the top 10 for binding according to MM-GBSA calculations pro cluster.
**Table S6.** Molecular docking MD-based analysis summary for CUB1-CUB2 domain/Ca\(^{2+}\)/GAG interaction.

| GAG | \(^{1} m, \epsilon\) | \(^{2} n\) | \(^{3} Size\) | \(^{4} \Delta G\) kcal/mol | Top \(^{5}\) MM-GBSA 10 residues for GAG binding | Polarity |
|-----|-----------------|-------------|-----------------|-----------------|---------------------------------|---------|
| HP, dp2 | 4, 1 | 1 | 10 | -14.0 ± 10.5, -46.9 ± 6.3, -4.6 ± 11.1 | R231, K234, R107, L120, Q254, R214, V256, L108, V121, G109 | 10/0 |
| | | | 2 | -16.1 ± 8.4, -28.6 ± 10.4, -20.2 ± 8.4 | R107, R231, K234, R110, Q106, G109, R214, Y212, G233, R130 | 6/0 |
| | | | 3 | -21.1 ± 7.0, -27.0 ± 7.8, -15.9 ± 13.5 | K234, R107, Q106, Y212, G233, F221, L232, R231, L108, S219 | 5/0 |
| HP, dp4 | 3, 2 | 1 | 7 | -13.6 ± 9.2, 1.4 ± 9.1, 6.9 ± 10.0 | R231, K234, R107, Q106, R91, G233, R110, R214, R130, R230 | 7/0 |
| | | | 2 | -42.1 ± 14.2, -31.1 ± 9.5, -40.4 ± 11.6 | R231, R130, R110, R91, K234, Q106, Y212, R107, R214, G233 | 6/0 |
| | | | 3 | -14.4 ± 10.9, -0.4 ± 5.6, -29.9 ± 8.8 | K234, R231, R107, R110, T211, F235, Y212, G233, Q106, C236 | 5/0 |
| HP, dp6 | 2, 2.45 | 1 | 17 | -14.4 ± 17.0, -31.9 ± 9.4, -21.1 ± 18.7 | R130, R91, R231, K234, Y212, R214, R107, R230, Q106, R110 | 17/0 |
| | | | 2 | -31.3 ± 11.1, -31.2 ± 12.2, -41.4 ± 7.8 | R110, K234, R130, R231, Y212, R214, G109, R107, R91, K61 | 9/0 |
| | | | 3 | -51.3 ± 9.9, -42.2 ± 19.4, -29.0 ± 11.7 | R91, R231, K234, Q106, R107, R214, K61, G109, R110, R230 | 3/0 |

\(^{1}\) DBSCAN parameters \(m\), the minimal neighborhood size and \(\epsilon\), neighborhood search radius [33]; \(^{2}\) Cluster number; \(^{3}\) Cluster size; \(^{4}\) Free energy of binding obtained by MM-GBSA; \(^{5}\) Residues identified in the top 10 for binding according to MM-GBSA calculations per cluster.
Table S7. Molecular docking MD-based analysis summary for PCPE-1 UNRES models/Ca\(^{2+}\)/GAG interaction.

| Structure | 1\(m, \epsilon\) | 2\# Size | 3\(\Delta G\) kcal/mol | 4Top\(_{\text{MM-GBSA}}\) 10 residues for GAG binding | 5Polarity |
|-----------|----------------|-----------|------------------------|--------------------------------|-----------|
| MODEL 1   | 2, 3.0         | 1 6       | -78.0 ± 20.2           | K393, R324, R323, K394, R288, K287, K320, M392, C322, K271 | 3/3       |
|           |                | 2 3       | -99.1 ± 20.0           | R323, R288, K320, R348, R324, K393, K279, K287, R275, C389 | 2/1       |
|           |                | 3 3       | -120.8 ± 13.1          | R323, R275, R288, R324, K287, K393, K271, R422, L273, R348 | 2/1       |
| MODEL 2   | 3, 2.95        | 1 6       | -107.2 ± 11.2          | K394, R348, K393, K295, K293, R324, M392, V347, M346, R422 | 3/3       |
|           |                | 2 4       | -84.3 ± 11.2           | K295, R348, K394, K293, K393, K299, V347, K305, L296, V294 | 4/0       |
|           |                | 3 4       | -49.6 ± 15.0           | K295, R288, K293, K344, K204, V294, R348, K299, K394, K287 | 4/0       |
| MODEL 3   | 2, 3.2         | 1 13      | -6.6 ± 11.1            | R275, K165, K271, K305, R288, K287, L273, Q403, P274, N407 | 7/6       |
|           |                | 2 3       | -37.5 ± 23.9           | R275, K271, K165, R162, K287, K293, L273, K295, P274, R288 | 2/1       |
|           |                | 3 3       | -68.9 ± 12.6           | K287, K271, K165, R288, K293, R275, K305, K295, K279, T290 | 2/1       |

DBSCAN parameters \(m\), the minimal neighborhood size and \(\epsilon\), neighborhood search radius [33]; 2 Cluster number; 3 Cluster size; 4 Free energy of binding obtained by MM-GBSA; 5 Residues identified in the top 10 for binding according to MM-GBSA calculations per cluster ordered by the impact (starting from the most favourable one). 6 The polarity of a GAG binding pose was defined as its preferred orientation in relation to the reducing and non-reducing end.
Table S8. Fragment-based molecular docking MD analysis summary for PCPE-1 UNRES models/Ca\(^{2+}\)/HP dp11 interaction.

| MODEL 1, HP dp11 | Pose number | Free energy of binding obtained by MM-GBSA | Residues identified in the top 10 for binding according to MM-GBSA calculations per cluster ordered by the impact (starting from the most favourable one) |
|------------------|-------------|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                  | 1           | -144.6±25.7                                 | R323, R324, K287, R275, K394, R348, K393, K271, L273, R288                                                                                                                                  |
|                  | 2           | -115.5±22.4                                 | K393, R323, K394, R324, R275, K287, K395, R348, M392, R288                                                                                                                                  |
|                  | 3           | -139.9±17.6                                 | R323, R348, K279, K394, R324, R275, K393, R288, K344, R422                                                                                                                                  |
|                  | 1           | -105.0±28.5                                 | R422, K295, R288, K393, K293, K394, K344, R348, R324, K436                                                                                                                                  |
|                  | 2           | -121.1±22.5                                 | R422, K394, R324, K393, K293, K287, K344, R345, K295, K320                                                                                                                                  |
|                  | 3           | -103.9±18.3                                 | K394, K295, K293, K393, R422, R348, R324, K287, V294, R275                                                                                                                                  |
|                  | 1           | -105.0±28.5                                 | R422, K295, R288, K393, K293, K394, K344, R348, R324, K436                                                                                                                                  |
|                  | 2           | -121.1±22.5                                 | R422, K394, R324, K393, K293, K287, K344, R345, K295, K320                                                                                                                                  |
|                  | 3           | -103.9±18.3                                 | K394, K295, K293, K393, R422, R348, R324, K287, V294, R275                                                                                                                                  |
|                  | 1           | -66.9±16.4                                  | R422, K394, R324, R323, N424, K393, P423, Q427, K320, K387                                                                                                                                  |
|                  | 2           | -111.4±19.1                                 | K393, R422, K394, K344, R345, R348, V396, R324, G395, K436                                                                                                                                  |
|                  | 3           | -90.9±26.9                                  | R324, K393, K344, K394, R422, R348, R323, K320, M392, T325                                                                                                                                  |
|                  | 1           | +157.1±18.3                                 | R323, K287, K271, R275, K165, K320, R288, K393, R324, K394                                                                                                                                  |
|                  | 2           | +151.4±24.5                                 | R348, R324, R323, R275, K393, K394, K287, K320, K271, R288                                                                                                                                  |
|                  | 3           | +110.4±16.6                                 | R324, K393, K344, K394, R422, R348, R323, K320, M392, T325                                                                                                                                  |
|                  | 1           | +135.3±33.6                                 | R288, K394, K393, R422, R348, K436, R435, K287, K295, K299                                                                                                                                  |
|                  | 2           | +130.7±26.3                                 | K293, R422, R348, R324, K393, K295, K394, K287, R288, K299                                                                                                                                  |
|                  | 3           | +141.2±21.7                                 | R324, K394, K393, R422, R323, R348, K295, K299, K293, K287                                                                                                                                  |
|                  | 1           | +52.1±26.8                                  | K287, K299, K271, K279, R288, R275, K293, K295, R162, K165                                                                                                                                  |
|                  | 2           | +99.0±33.0                                  | K165, K271, R275, K295, K293, K287, P292, K204, R288, K279                                                                                                                                  |
|                  | 3           | -69.7±13.8                                  | R288, K287, R275, K293, K279, K295, T290, R323, K204, K165                                                                                                                                  |

1 Pose number; 2 Ca\(^{2+}\) presence; 3 Free energy of binding obtained by MM-GBSA; 4 Residues identified in the top 10 for binding according to MM-GBSA calculations per cluster ordered by the impact (starting from the most favourable one).
Figure S1. Models 1, 2, 3 (upper panel). NTR domain: green; CUB1-CUB2: red; the interdomain linker between the CUB2 and NTR domains: black. Positive electrostatic potential isosurfaces of PCPE-1 models (2.0 kcal/mol e⁻¹) in the absence of Ca²⁺ ions obtained by PBSA calculations.
Figure S2. Molecular docking and MM-GBSA results for CUB1-CUB2-GAG complexes. The structure of the CUB1-CUB2 domains is shown in cartoon representation at the top. For each GAG, the analyzed clusters of docking solutions are shown in blue, red, yellow and green (from the most to the least populated cluster); the top 10 residues binding to GAGs according to MM-GBSA calculations averaged per GAG are highlighted in red surface, respectively.
Figure S3. CUB1-CUB2 amino acid residues identified in the top 10 for binding GAGs according to MM-GBSA calculations per cluster are labeled as an asterisk.
Figure S4. Molecular docking and MM-GBSA results for NTR/Ca\(^{2+}\)/HP systems. For each length of HP, the analyzed clusters of docking solutions are shown in blue, red and yellow (from the most to the least populated); the top 10 residues for binding GAGs according to MM-GBSA calculations averaged pro GAG are highlighted in red surface, respectively.

Figure S5. Molecular docking and MM-GBSA results for CUB1-CUB2/Ca\(^{2+}\)/HP systems. For each length of HP, the analyzed clusters of docking solutions are shown in blue, red and yellow (from the most to the least populated); the top 10 residues for binding GAGs according to MM-GBSA calculations averaged pro GAG are highlighted in red surface, respectively.
Figure S6. Molecular docking results for the models of full-length PCPE-1 protein in the absence and presence of Ca^{2+} ions and HP dp6. The clusters of docking solutions are shown in blue, red and yellow (from the most to the least populated clusters). NTR domain: green; CUB1-CUB2: red; the interdomain linker between the CUB2 and NTR domains: black.
Figure S7. Molecular docking results for the models of full PCPE-1 protein in the absence (in blue) and presence (in red) of Ca$^{2+}$ ions and HP dp11 corresponding to the most favourable free binding energies.