Selective Disruption of Survivin’s Protein-Protein Interactions: A Supramolecular Approach Based on Guanidiniocarbonylpyrrole

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1. General Information

Water was purified via a TKA MicroPure ultrapure water system. All solvents were distilled before use. All commercial chemicals were used as received unless otherwise specified. Reactions were monitored by TLC on silica gel plates (Macherey-Nagel POLYGRAM SIL G/UV254). Spots were visualized by UV light (254 nm and 366 nm). For lyophilisation a Christ Alpha 1-4 LDplus freeze dryer was used. The pH was adjusted with a pH-meter 766 Calimatic from Knick. Melting points were measured with a Büchi Melting-Point B-540 apparatus with open end glass capillary tubes. The melting points are not corrected. FT-IR measurements were performed with a Jasco FT/IR-430 spectrometer. The NMR spectra were recorded with Bruker DMX 300, DRX 500 or AVHD 600 spectrometers. All NMR measurements were performed at room temperature using DMSO-d$_6$ as solvent. The chemical shifts are relative to the signals of DMSO-d$_6$ ($\delta$ $^1$H = 2.50 ppm and $\delta$ $^{13}$C = 39.5 ppm). The apparent coupling constants are given in Hertz. The description of the fine structure means: s = singlet, br. s = broad singlet, d = doublet, t = triplet, m = multiplet.
2. Computational Details

**Fig. S1.** Examples of calculated structures of \( L_{\text{HIS}} \) and \( L_{\text{NES}} \)

The coordinates of Survivin with PDB ID:1XOX were taken from the Protein Data Bank.[1-3]

**Fig. S2.** Front and back view representations of the histone binding site and the NES region of human Survivin, shown as red and green surfaces, respectively. The tetrahedrally coordinated \( \text{Zn}^{2+} \) is shown as a blue sphere.

**Methods**

**Advanced conformational sampling of ligands in water**

In all cases, three replicas (250 ns each) of Gaussian accelerated Molecular Dynamics (GaMD) simulations were performed[4,5] for an extended sampling of the conformations of \( L_{\text{HIS}} \) and \( L_{\text{NES}} \) in water. The biasing potential was equilibrated in two steps: first, 5 ns of classical MDs in which the potential energy distribution of the system is monitored. Then, during 20 ns, the biasing potential is equilibrated by fixing an energy threshold at the maximum value of the potential energy and successively updating this value after every step of the simulation. The constant of the harmonic bias is concurrently equilibrated by keeping the maximum standard deviation of the biasing potential at 10 kT units. Along the 250 ns of production run, an average bias of 7.3 kcal/mol was applied at every step of the trajectory. The biasing potential creates a smoothed potential energy surface, which allows sampling dynamic events that would need hundreds of nanoseconds of conventional MD simulation. Here, the GaMD advanced sampling technique is used to perform an extensive conformational sampling of \( L_{\text{HIS}} \) and \( L_{\text{NES}} \) in solution. This allowed us to extract the most populated clusters of structures in each case (Figures S3 and S4).
Fig. S3. Representative structures of the six largest clusters, cluster-0 to cluster-5 (C0 to C5), of \textbf{L}\textsubscript{HIS} from GaMD sampling in water.

Fig. S4. Representative structures of the six largest clusters, cluster-0 to cluster-5 (C0 to C5), of \textbf{L}\textsubscript{NES} from GaMD sampling in water.

**Protein-ligand docking**

Docking simulations of \textbf{L}\textsubscript{HIS} and \textbf{L}\textsubscript{NES} with Survivin were performed using LeDock.\cite{6,7} All parameters were set to default for conformational sampling and docking scores were calculated by the default scoring function.\cite{6,7} The dimension of the docking box was fixed in order to contain the complete protein, VMD was used to measure the protein dimensions.\cite{8} The docking simulations followed by cluster analysis, as implemented in LeDock, allowed us to extract three out of the top ten most favoured binding poses (clusters) of the \textbf{L}\textsubscript{HIS} and \textbf{L}\textsubscript{NES} ligands on the monomer form of Survivin (Figure S5). Further, the selected poses were relaxed using MD simulations.
Fig.S5. Representation of the favored binding sites of \( \mathbf{L_{\text{HIS}} \text{ and } L_{\text{NES}}} \) in Survivin (C1, C2 and C3 are representative structures in each case). Remarkably, for \( \mathbf{L_{\text{HIS}}} \) two out of the three top clusters populate the Histone H3 binding site, while for \( \mathbf{L_{\text{NES}}} \) two clusters are found in the NES region.

**Molecular dynamics simulations of the bound structures**

The NAMD 2.12 software \(^9\) with the CHARMM36m force field \(^10\) was used to perform the MD simulations. The parameters and topologies for ligands \( \mathbf{L_{\text{HIS}}} \) and \( \mathbf{L_{\text{NES}}} \) were obtained using SwissParam.\(^{11}\) The protein-ligand complexes were placed in a solvent box with 20 Å of padding in the three dimensions. TIP3P was used for the water molecules.\(^{12}\) Counter ions were added for neutralization in each system. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method.\(^{13}\) Production runs were carried out at the NPT ensemble (1 atm and 300 K) using Langevin dynamics.\(^{14}\) This simulation set up was also used for the GaMD calculations with the ligands in solution, the only difference there was that the boxes were built with a padding of 15 Å.

For the four complexes, MD simulations (five replicas of 100 ns each) were carried out to further explore the binding modes of each ligand in the two sites. The resulting 500 ns of simulation were combined and clustered to extract the most representative binding modes for each ligand (Figure S6).
Fig. S6. \textbf{L}_{\text{HIS}}\text{ and }\textbf{L}_{\text{NES}}\text{ binding modes at the NES and the Histone H3 binding site. Ligands are represented in licorice. Hydrogens are omitted for clarity. Aspartate and glutamate residues forming salt bridges with the ligands are represented in CPK and highlighted with black labels.}

**Binding free energy calculations**

The Central Limit FEP (CL-FEP) free energy perturbation approach was used to estimate the affinity of \textbf{L}_{\text{HIS}}\text{ and }\textbf{L}_{\text{NES}}\text{ for the above-discussed binding sites.}^{[15]}\text{ CL-FEP is an end-state approach, therefore only sampling of bound (complex and bulk solvent boxes) and unbound (ligand and protein boxes) states is needed. In this context, six replicas of }\text{~40 ns molecular dynamic simulations were performed for sampling each box, thus completing 240 ns of sampling in all subsystems where the energy was obtained every 5 ps. The construction of the simulation boxes for all the systems, as well as of the NAMD configuration files was carried out with the preparation module of the web server CLFEP-GUI (https://clfep.zmb.uni-due.de/). The server ensures the use of the same number of water molecules for all subsystems (complex, ligand, protein, and bulk solvent). A salt concentration of 0.15 M was fixed in all the simulation boxes, balancing the number of charges in each box. The protein was restrained by means of harmonic wall potentials based on the RMSD with the initial structure (walls located at RMSD = 0 Å and RMSD = 5 Å). Additionally, the distance between the center of masses of the ligand and the binding site was also restrained (walls located at distance = 0 Å and distance = 5 Å).

The analyses of the outcome from the sampling simulations were conducted with the Analysis module of CLFEP-GUI. Fifteen checkpoints were used to study the convergence of CL-FEP. All analysis parameters were used as the default of the server. For each checkpoint, CL-FEP assesses the estimator convergence by three criteria: i) the achievement of a Gaussian energy distribution among multiple bootstrap energy means (otherwise, WARNING1 is issued). ii) The
expected standard deviation of the free energy estimation is below kT (otherwise, WARNING2 is issued). iii) The actual standard deviation among replicas of the evaluation of the estimator is below kT (otherwise, WARNING3 is issued). All checkpoints with converged results were averaged to obtain the final free energy estimation. In addition, for assessing the sampling convergence, CLFEP computes the standard deviation among the individual converged checkpoints and compares it to nkT, where ‘n’ is the squared root of the number of converged checkpoints. If the deviation is below this threshold the convergence of the estimation is accepted (otherwise, WARNING4 is issued). Tables S1-S4 summarize the convergence analyses for each system.

Table S1. Thermodynamic and convergence parameters obtained for all the checkpoints in the system L\textsubscript{HIS} - NES using the second order cumulant estimator (C2). ISR: Importance sampling ratio. RMSE: Root mean squared error. The final average value corresponds to the ISR-weighted average among the converged checkpoints.

| Checkpoint | ΔG (kcal/mol) | RMSE (kcal/mol) | ISR (%) | Convergence |
|------------|---------------|-----------------|---------|-------------|
| 1 (30)     | -4.01         | 1.01            | 7.40    | WARNING3    |
| 2 (35)     | -5.88         | 0.79            | 19.18   | WARNING3    |
| 3 (40)     | -3.70         | 0.74            | 17.92   | WARNING3    |
| 4 (45)     | -4.03         | 0.57            | 20.11   | OK          |
| 5 (50)     | -1.84         | 0.80            | 30.41   | WARNING3    |
| 6 (55)     | -4.71         | 0.56            | 29.37   | OK          |
| 7 (60)     | -4.06         | 0.82            | 19.10   | WARNING3    |
| 8 (65)     | -4.12         | 0.69            | 35.96   | WARNING3    |
| 9 (70)     | -2.90         | 0.60            | 51.63   | WARNING3    |
| 10 (75)    | -3.75         | 0.74            | 40.00   | WARNING3    |
| 11 (80)    | -3.43         | 0.54            | 35.73   | OK          |
| 12 (85)    | -4.42         | 0.56            | 28.57   | OK          |
| 13 (90)    | -3.80         | 0.58            | 30.27   | OK          |
| 14 (95)    | -3.72         | 0.62            | 35.14   | WARNING3    |
| 15 (100)   | -3.66         | 0.59            | 42.69   | OK          |
| **Average**| **-3.96**     | **0.57**        |         | **OK**      |
Table S2. Thermodynamic and convergence parameters obtained for all the checkpoints in the system $L_{\text{HIS}}$ – Histone H3 binding site using the second order cumulant estimator (C2). ISR: Importance sampling ratio. RMSE: Root mean squared error. The final average value corresponds to the ISR-weighted average among the converged checkpoints.

| Checkpoint | $\Delta G$ (kcal/mol) | RMSE (kcal/mol) | ISR (%) | Convergence  |
|------------|------------------------|-----------------|---------|--------------|
| 1 (30)     | -8.80                  | 1.17            | 15.13   | WARNING3     |
| 2 (35)     | -6.20                  | 0.93            | 17.17   | WARNING3     |
| 3 (40)     | -6.52                  | 0.75            | 21.01   | WARNING3     |
| 4 (50)     | -6.02                  | 0.75            | 24.07   | WARNING3     |
| 5 (60)     | -8.11                  | 0.70            | 17.38   | WARNING3     |
| 6 (65)     | -6.46                  | 0.72            | 24.18   | WARNING3     |
| 7 (70)     | -6.90                  | 0.67            | 29.07   | WARNING3     |
| 8 (85)     | -7.67                  | 0.63            | 27.66   | WARNING3     |
| 9 (90)     | -6.63                  | 0.59            | 35.99   | OK           |
| 10 (95)    | -7.62                  | 0.55            | 25.23   | OK           |
| 11 (100)   | -6.71                  | 0.59            | 32.61   | WARNING3     |
| 12 (95)    | -7.37                  | 0.57            | 43.23   | OK           |
| 13 (85)    | -7.67                  | 0.57            | 32.22   | OK           |
| 14 (90)    | -6.96                  | 0.73            | 46.84   | WARNING3     |
| 15 (100)   | -7.02                  | 0.68            | 47.32   | WARNING3     |
| **Average** | **-7.29**              | **0.57**        |         | **OK**       |

Table S3. Thermodynamic and convergence parameters obtained for all the checkpoints in the system $L_{\text{NES}}$ – NES using the second order cumulant estimator (C2). ISR: Importance sampling ratio. RMSE: Root mean squared error. The final average value corresponds to the ISR-weighted average among the converged checkpoints.

| Checkpoint | $\Delta G$ (kcal/mol) | RMSE (kcal/mol) | ISR (%) | Convergence  |
|------------|------------------------|-----------------|---------|--------------|
| 1 (30)     | -16.15                 | 0.68            | 7.93    | WARNING2     |
| 2 (35)     | -14.38                 | 0.64            | 8.55    | WARNING3     |
| 3 (40)     | -13.26                 | 0.64            | 7.69    | WARNING3     |
| 4 (50)     | -12.17                 | 0.52            | 27.85   | OK           |
| 5 (60)     | -14.54                 | 0.78            | 12.96   | WARNING3     |
| 6 (65)     | -12.71                 | 0.63            | 41.78   | WARNING3     |
| 7 (70)     | -13.89                 | 0.73            | 26.27   | WARNING3     |
| 8 (85)     | -14.68                 | 0.65            | 21.90   | WARNING3     |
| 9 (90)     | -13.80                 | 0.57            | 32.63   | OK           |
| 10 (95)    | -13.66                 | 0.74            | 31.55   | WARNING3     |
| 11 (100)   | -14.13                 | 0.56            | 48.18   | OK           |
| 12 (95)    | -14.09                 | 0.73            | 36.39   | WARNING3     |
| 13 (90)    | -14.64                 | 0.54            | 30.22   | OK           |
| 14 (95)    | -13.55                 | 0.53            | 24.44   | OK           |
| 15 (100)   | -13.83                 | 0.75            | 30.92   | WARNING3     |
| **Average** | **-13.74**             | **0.83**        |         | **OK**       |
**Table S4.** Thermodynamic and convergence parameters obtained for all the checkpoints in the system LNES – Histone H3 binding site using the second order cumulant estimator (C2). ISR: Importance sampling ratio. RMSE: Root mean squared error. The final average value corresponds to the ISR-weighted average among the converged checkpoints.

| Checkpoint | ΔG (kcal/mol) | RMSE (kcal/mol) | ISR (%) | Convergence |
|------------|---------------|-----------------|---------|-------------|
| 1 (30)     | -1.08         | 0.66            | 3.36    | WARNING2    |
| 2 (35)     | -3.44         | 0.84            | 5.63    | WARNING3    |
| 3 (40)     | -4.45         | 0.72            | 11.97   | WARNING3    |
| 4 (45)     | -4.59         | 0.99            | 22.42   | WARNING3    |
| 5 (50)     | -4.79         | 0.96            | 16.85   | WARNING3    |
| 6 (55)     | -4.61         | 0.68            | 14.07   | WARNING3    |
| 7 (60)     | -4.71         | 0.55            | 16.72   | OK          |
| 8 (65)     | -4.46         | 0.69            | 17.47   | WARNING3    |
| 9 (70)     | -5.56         | 0.54            | 23.04   | OK          |
| 10 (75)    | -3.95         | 0.56            | 19.56   | OK          |
| 11 (80)    | -2.85         | 0.67            | 26.95   | WARNING3    |
| 12 (85)    | -5.10         | 0.62            | 19.12   | WARNING3    |
| 13 (90)    | -2.96         | 0.60            | 26.18   | WARNING3    |
| 14 (95)    | -3.87         | 0.57            | 42.14   | OK          |
| 15 (100)   | -3.95         | 0.61            | 39.35   | WARNING3    |
| **Average** | **-4.41**     | **0.68**        |         | **OK**      |
3. Synthesis

The synthesis of building block A was previously reported by us.\textsuperscript{17} The synthesis of the introduced GCP binding motif E is described by known literature.\textsuperscript{18}

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\includegraphics[width=0.4\textwidth]{Scheme_S1_A}};\node (B) at (0,1) {\includegraphics[width=0.4\textwidth]{Scheme_S1_B}};\node (C) at (0,2) {\includegraphics[width=0.4\textwidth]{Scheme_S1_C}};\node (D) at (0,3) {\includegraphics[width=0.4\textwidth]{Scheme_S1_D}};\node (E) at (0,4) {\includegraphics[width=0.4\textwidth]{Scheme_S1_E}};\node (F) at (0,5) {\includegraphics[width=0.4\textwidth]{Scheme_S1_F}};\node (G) at (0,6) {\includegraphics[width=0.4\textwidth]{Scheme_S1_G}};\node (H) at (0,7) {\includegraphics[width=0.4\textwidth]{Scheme_S1_H}};\end{tikzpicture}
\end{center}

\textbf{Scheme S1:} Synthesis of the tetra cationic ligand $L_{\text{HIS}}$ and ligand $L_{\text{NES}}$. 
GCP-L-DAP(GCP)-OH B

GCP-L-Dap(GCP)-OMe A (0.89 g, 1.32 mmol) were dissolved in a mixture of THF (10 mL) and H₂O (5 mL) followed by addition of LiOH (31.6 mg, 1.32 mmol). The resulting reaction mixture was stirred for 6 h. Subsequently, THF was removed under reduced pressure and the pH set to 4 by addition of 1 M citric acid. The resulting precipitate was filtrated and washed with water. The solid was dried by lyophilisation leading to the product B (0.81 g, 1.18 mmol, 93%) as a white powder.

**Molecular Formular:** C₂₇H₃₆N₁₀O₁₀; **Molecular Mass:** 660.65 g/mol; **Mp:** 256.0 °C (decomposition); **¹H NMR:** (600 MHz, DMSO-d₆) δ [ppm] = 1.45 (s, 18H, Boc-C₃H₃), 3.54 (m, 1H, ß-C₃H₂), 3.77 (m, 1H, ß-C₃H₂), 4.56 (q, 1 H, ³J(H, H) = 6.86 Hz, α-CH ), 6.76 (m, 4H, Pyrrole-CH), 8.57-8.68 (m, 4H, NH), 9.32 (s, 2H, NH), 11.41 (br. s, 4H, Gua-NH); **¹³C-NMR:** (150 MHz, DMSO-d₆) δ [ppm] = 27.6, 27.8, 30.4, 40.1, 40.2, 52.3, 112.2, 112.4, 113.6, 158.4, 159.6, 160.1, 171.9; **FT-IR:** (ATR) ν [cm⁻¹] = 3263, 2295, 1726, 1691, 1630, 1549, 1531, 1468, 1392, 1369, 1236, 1142, 1047, 839, 754; **HR-MS:** (pos. ESI, MeOH) m/z = 661.2664 ([M+H]⁺, calc.: 661.2689).

Protected Ligand C

GCP-L-DAP(GCP)-OH B (0.59 g, 0.89 mmol) and PyBOP (0.51 g, 0.98 mmol) were solved in DMF (10 mL) after addition of NMM (165 µL, 1.48 mmol) the reaction mixture was stirred for 15 min. Then Ethylenediamine (19.7 µL, 0.29 mmol) was added and the mixture stirred for three days. The reaction mixture was precipitated in water (20 mL) and dried under vacuo. The crude product was purified by column chromatography (SiO₂, DCM/ACN/MeOH = 9/1/0.5) to give C (275 mg, 0.20 mmol, 69%) as a white solid.

**Molecular Formular:** C₅₆H₇₆N₂₂O₁₈; **Molecular Mass:** 1345.36 g/mol; **Mp:** 247.0-248.0 °C (decomposition); **¹H NMR:** (600 MHz, DMSO-d₆) δ [ppm] = 1.45 (s, 36H, Boc-C₃H₃), 3.13 (m, 4H, CH₂), 3.56-3.66 (m, 4H, ß-CH₂), 4.55 (m, 2 H, α-CH ), 6.77 (m, 8H, Pyrrole-CH), 8.14 (s, 2H, NH), 8.47-8.53 (m, 8H, NH), 9.32 (s, 4H, NH), 10.32-11.37 (m, 8H, NH); **¹³C-NMR:** (150 MHz, DMSO-d₆) δ [ppm] = 27.8, 38.3, 40.1, 40.7, 53.4, 112.2, 112.6, 113.8, 158.4, 159.6, 160.3, 169.9; **FT-IR:** (ATR) ν [cm⁻¹] = 3263, 2979, 2297, 1728, 1630, 1547, 1529, 1468, 1392, 1369, 1236, 1142, 1047, 839, 754; **HR-MS:** (pos. ESI, MeOH) m/z = 673.2910 ([M+2H]²⁺, calc.: 673.2927).
Ligand L\textsubscript{NES}:

\begin{center}
\includegraphics[width=0.5\textwidth]{ligand_lnes.png}
\end{center}

C (100 mg, 0.07 mmol) was dissolved in DCM (5 mL) followed by addition of TFA (3 mL). The reaction mixture was stirred for 5 h, then the solvent was removed under reduced pressure. The crude product was purified by MPLC on C18 reversed-phase silica gel (gradient 5% → 100% MeOH/H\textsubscript{2}O in 120 min, 0.1% TFA) to give L\textsubscript{NES} as TFA salt (98.9 mg, 0.07 mmol, 95%) as white solid. The hydrochloric salt L\textsubscript{NES} was obtained as white solid, by addition of HCl (0.1 M, 2 mL) and lyophilization (three times).

Molecular Formular: \textit{C}_{36}\textit{H}_{48}\textit{Cl}_{4}\textit{N}_{22}\textit{O}_{10};  
Molecular Mass: 1090.72 g/mol;  
\textit{Mp}: 259.4 °C (decomposition);  
\textbf{\textit{1H NMR}}: (600 MHz, DMSO-d\textsubscript{6}) \(\delta\) [ppm] = 3.10-3.17 (m, 4H, CH\textsubscript{2}), 3.57 (m, 2H, ß-C\textsubscript{H}\textsubscript{2}), 3.69 (m, 2H, ß-C\textsubscript{H}\textsubscript{2}), 3.80 (m, 2H, CH\textsubscript{2}), 3.85 (m, 2H, CH\textsubscript{2}), 4.62 (q, 2H, ß-J(H, H) = 6.79 Hz, ß-C\textsubscript{H}), 6.87-6.92 (m, 4H, Pyrrole-CH\textsubscript{2}), 7.03-7.08 (m, 4H, Pyrrole-CH\textsubscript{2}), 7.23 (s, 2H, NH), 8.41-8.66 (m, 18H, NH & Gua-NH\textsubscript{2}), 8.75 (d, 2H, ß-J(H, H) = 7.67 Hz, NH), 11.87 (m, 4H, NH), 12.35-12.52 (m, 4H, NH);  
\textbf{\textit{13C NMR}}: (150 MHz, DMSO-d\textsubscript{6}) \(\delta\) [ppm] = 38.3, 40.1, 40.7, 53.2, 112.9, 113.4, 115.7, 115.8, 115.8, 125.5, 125.5, 125.6, 132.4, 132.5, 155.3, 155.4, 159.1, 159.5, 159.6, 159.7, 169.6;  
\textbf{\textit{FT-IR}}: (ATR) \(\tilde{\nu}\) [cm\textsuperscript{-1}] = 3323, 2285, 1691, 1641, 1570, 1477, 1433, 1286, 1248, 1200, 1149, 1072, 1004, 957, 852, 814, 752, 665;  
\textbf{HR-MS}: (pos. ESI, MeOH) m/z = 315.7915 ([M+3H]\textsuperscript{3+}, calc.: 315.7943).

GCP-L-Lys-(GCP)-OMe F:

\begin{center}
\includegraphics[width=0.5\textwidth]{gcp_l_lys_gcp_ome_f.png}
\end{center}

H-Lys-OMe 2 HCl D (0.49 g, 2.1 mmol) was solved in DMF (5 mL) and NMM (0.7 mL, 6.3 mmol), in additon, GCP E (2.6 g, 6.5 mmol) was added and the reaction was cooled to 0°C and stirred for 30 min. Subsequently, HOBt (170 mg, 1.26 mmol) and EDC \cdot HCl (1.28 g, 6.7 mmol) were added and the reaction mixture was stirred for additional 30 min at 0°C, than the cool bath was removed and stirred overnight. The reaction mixture was dropped in H\textsubscript{2}O (100 mL) and the precipitate was filtered. The resulting crude product was dried under reduced pressure and purified by column chromatography (SiO\textsubscript{2}, DCM/ACN/MeOH = 9/1/0.5) to obtain F (1.09 g, 1.52 mmol, 72%) as a white solid.

Molecular Formular: \textit{C}_{31}\textit{H}_{44}\textit{N}_{10}\textit{O}_{10};  
Molecular Mass: 716.75 g/mol;  
\textit{Mp}: 146.1 °C (decomposition);  
\textbf{\textit{1H NMR}}: (600 MHz, DMSO-d\textsubscript{6}) \(\delta\) [ppm] = 1.45 (m, 22H, Boc-CH\textsubscript{2} & Lys-CH\textsubscript{2}),...
1.76-1.81 (m, 2H, Lys-C\H_2), 3.22 (q, 2H, 3J(H, H) = 6.47 Hz, ɛ-C\H_2), 3.63 (s, 3H, O-C\H_3), 4.40 (m, 1H, α-C\H), 6.75-6.83 (m, 4H, Pyrrole-C\H), 8.33 (t, 1H, 3J(H, H) = 5.48 Hz, N\H), 8.60 (m, 3H, NH), 9.32 (s, 2H, NH), 10.84-11.51 (m, 4H, N\H); 13C NMR: (150 MHz, DMSO-d_6) δ [ppm] = 23.1, 27.8, 28.7, 30.4, 38.4, 40.1, 51.9, 52.1, 111.6, 112.9, 125.6, 158.4, 159.5, 159.7, 172.7;
FT-IR: (ATR) ̃v [cm⁻¹] = 3373, 2979, 2267, 1786, 1726, 1691, 1546, 1529, 1468, 1392, 1369, 1286, 1232, 1142, 1045, 841, 779, 754;
HR-MS: (pos. ESI, MeOH) m/z = 717.3303 ([M+H]^+ , calc.: 717.3315).

GCP-L-Lys-(GCP)-OH G

GCP-L-Lys(GCP)-OMe F (0.94 g, 1.31 mmol) were dissolved in a mixture of THF (20 mL) and H_2O (10 mL) followed by addition of LiOH (31.4 mg, 1.31 mmol) and stirred for 6 h. Then THF was removed under reduced pressure and the pH adjusted to 4 with addition of 1 M citric acid. The resulting precipitate was filtrated and washed with water. The solid was dried by lyophilisation leading to the product G (0.87 g, 1.23 mmol, 94%) as a white powder.

Protected Ligand H

GCP-L-Lys(GCP)-OH G (0.62 g, 0.88 mmol) and Ethylenediamine (19.6 µL, 0.29 mmol) were solved in DMF (10 mL) after addition of NMM (179 µL, 1.61 mmol) the reaction mixture was stirred for 30 min at 0 °C. Subsequently, HOBt (23 mg, 0.18 mmol) and EDC · HCl (186 mg, 0.97 mmol) were added and the reaction mixture stirred for additional 30 min at 0 °C, then the cool bath was removed and stirred for three days. The reaction mixture was precipitated in water (50 mL) and dried under vacuo. The crude product was purified by column chromatography (SiO_2, DCM/ACN/MeOH = 9/1/0.5) to give H (261 mg, 0.18 mmol, 63%) as a white solid.
**Molecular Formula:** $C_{62}H_{88}N_{22}O_{18}$; **Molecular Mass:** 1429.52 g/mol; **Mp:** 235.5-272.3 °C (decomposition); **$^1$H NMR:** (600 MHz, DMSO-d$_6$) $\delta$ [ppm] = 1.25-1.58 (m, 44H, Boc-CH$_3$ & Lys-CH$_2$), 1.65-1.72 (m, 4H, Lys-CH$_2$), 3.11 (m, 4H, CH$_2$), 3.20 (m, 4H, $\varepsilon$-CH$_2$), 4.34 (m, 2H, $\alpha$-CH$_2$), 6.75-6.82 (m, 8H, Pyrrole-CH$_2$), 8.08 (m, 2H, NH), 8.31 (t, 2H, $^3$J(H, H) = 5.32 Hz, NH), 8.42 (m, 2H, NH), 8.56 (s, 4H, NH), 9.31 (s, 4H, NH), 10.84-11.63 (m, 8H, NH); **$^{13}$C NMR:** (150 MHz, DMSO-d$_6$) $\delta$ [ppm] = 23.2, 27.8, 28.9, 31.6, 38.3, 38.5, 40.1, 52.9, 111.6, 112.9, 158.4, 159.5, 171.9; **FT-IR:** (ATR) $\tilde{\nu}$ [cm$^{-1}$] = 3377, 2979, 2299, 1726, 1689, 1630, 1547, 1529, 1468, 1392, 1369, 1288, 1236, 1144, 1045, 841, 779, 754; **HR-MS:** (pos. ESI, MeOH) m/z = 715.3367 ([M+2H]$^{2+}$, calc.: 715.3396).

**Ligand LHIS**

$L$ (200 mg, 0.14 mmol) was dissolved in DCM (10 mL) followed by addition of TFA (6 mL). The reaction mixture was stirred for 5 h, then the solvent was removed under reduced pressure. The crude product was purified by MPLC on C18 reversed-phase silica gel (gradient 5% $\rightarrow$ 100% MeOH/H$_2$O in 120 min, 0.1% TFA) to give LHIS as TFA salt (195.4 mg, 0.13 mmol, 94%) as white solid. The hydrochloric salt LHIS was obtained as white solid, by addition of HCl (0.1 M, 2 mL) and lyophilization (three times).

**Molecular Formula:** $C_{42}H_{60}Cl_4N_{22}O_{10}$; **Molecular Mass:** 1174.89 g/mol; **Mp:** 257.4-281.6 °C (decomposition); **$^1$H NMR:** (600 MHz, DMSO-d$_6$) $\delta$ [ppm] = 1.31-1.37 (m, 4H, Lys-CH$_2$), 1.50 (m, 4H, Lys-CH$_2$), 1.65 (m, 2H, CH$_2$), 1.73 (m, 2H, Lys-CH$_2$), 3.11 (m, 4H, CH$_2$), 3.20 (m, 4H, $\varepsilon$-CH$_2$), 4.37 (m, 2H, $\alpha$-CH$_2$), 6.83-6.90 (m, 4H, Pyrrole-CH$_2$), 7.44-7.49 (m, 4H, Pyrrole-CH$_2$), 8.15 (m, 2H, NH), 8.21-8.83 (m, 20H, NH & Gua-NH$_2$), 11.87-11.93 (m, 4H, NH), 12.28 (s, 2H, NH), 12.50 (s, 2H, NH); **$^{13}$C NMR:** (150 MHz, DMSO-d$_6$) $\delta$ [ppm] = 23.1, 28.7, 31.7, 38.3, 38.6, 40.1, 53.0, 112.3, 113.6, 115.7, 115.9, 125.3, 125.6, 132.4, 132.9, 155.4, 158.9, 159.0, 171.7; **FT-IR:** (ATR) $\tilde{\nu}$ [cm$^{-1}$] = 3299, 2347, 2305, 1685, 1658, 1630, 1550, 1479, 1442, 1400, 1286, 1207, 1144, 1003, 852, 746, 696, 606; **HR-MS:** (pos. ESI, MeOH) m/z = 515.2329 ([M+2H]$^{2+}$, calc.: 515.2348).
4. NMR Spectra

Fig. S7: $^1$H-NMR spectrum of B (600 MHz, DMSO-$d_6$).

Fig. S8: $^1$H-NMR spectrum of C (600 MHz, DMSO-$d_6$).
Fig. S9: $^1$H-NMR spectrum of $L_{\text{NES}}$ (600 MHz, DMSO-$d_6$).

Fig. S10: $^1$H-NMR spectrum of F (600 MHz, DMSO-$d_6$).
Fig. S11: $^1$H-NMR spectrum of G (600 MHz, DMSO-$d_6$).

Fig. S12: $^1$H-NMR spectrum of H (600 MHz, DMSO-$d_6$).
Fig. S13: $^1$H-NMR spectrum of L$_{\text{HIS}}$ (600 MHz, DMSO-$d_6$).

Fig. S14: $^{13}$C-NMR spectrum of B (150 MHz, DMSO-$d_6$).
Fig. S15: $^{13}$C-NMR spectrum of C (150 MHz, DMSO-$d_6$).

Fig. S16: $^{13}$C-NMR spectrum of L$_{NES}$ (150 MHz, DMSO-$d_6$).
Fig. S17: $^{13}$C-NMR spectrum of F (150 MHz, DMSO-$d_6$).

Fig. S18: $^{13}$C-NMR spectrum of G (150 MHz, DMSO-$d_6$).
Fig. S19: $^{13}$C-NMR spectrum of H (150 MHz, DMSO-$d_6$).

Fig. S20: $^{13}$C-NMR spectrum of $L_{\text{His}}$ (150 MHz, DMSO-$d_6$).
5. Mass Spectra

Fig. S21: HR-ESI mass spectrum of B (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to B.

Fig. S22: HR-ESI mass spectrum of C (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to C.

Fig. S23: HR-ESI mass spectrum of L_{NES} (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to L_{NES}. 
**Fig. S24:** HR-ESI mass spectrum of F (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to F.

**Fig. S25:** HR-ESI mass spectrum of G (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to G.

**Fig. S26:** HR-ESI mass spectrum of H (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to H.
Fig. S27: HR-ESI mass spectrum of $L_{\text{HIS}}$ (positive ion mode, MeOH) and predicted mass spectrum of peaks which belongs to $L_{\text{HIS}}$. 
6. ITC measurements

The ITC measurements were performed with the MicroCal iTC200 system from GE Healthcare Life Sciences.

Fig. S28: ITC measurement of Survivin-120 (1 mM) with L_NES (0.1 mM) in water with HEPES (2.5 mM), NaCl (10 mM), MgCl₂ (1 mM) and TCEP (0.05 mM), pH = 6.5. A) Raw data from the single injections. B) Integrated peaks as well as the corresponding fit and the thermodynamic parameters.
Fig. S29: ITC measurement of Survivin-120 (1 mM) with L_{HIS} (0.1 mM) in water with HEPES (2.5 mM), NaCl (10 mM), MgCl₂ (1 mM) and TCEP (0.05 mM), pH = 6.5. A) Raw data from the single injections L_{NES}. B) Integrated peaks as well as the corresponding fit and the thermodynamic parameters.

7. Cultivation of eukaryotic cells

The adherent eukaryotic cell lines used in this work were cultivated in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% FCS and 1% Antibiotic-Antimycotic (Life Technologies) at 37 °C, 5% CO₂ and 90% relative humidity. They were passaged twice a week in a ratio of 1:20 to regulate cell density and to supply the cells with fresh growth medium. For this, growth medium was aspirated, and the cells were rinsed with 5 ml DPBS. Afterwards, 2 ml of TrypLE Express (Life Technologies) were added to the cells to allow enzymatic detachment from the cell culture flask. The cells were incubated on a heating plate until all of them were detached before 8 ml of new growth medium were added. 0.5 ml of the cell suspension were then added to a new culture flask together with 9.5 ml of fresh growth medium.
8. Proximity ligation assay

Generally, the PLA enables the analysis of endogenous protein-protein interactions inside a cell. Briefly, primary antibodies bind to two potentially interacting targets, in our case Survivin and its export receptor Crm1 or Survivin and Histone H3. These antibodies are recognized by secondary antibodies, which are conjugated to a matched pair of short single-stranded oligonucleotides (PLA probes). If the two targets interact and are in close proximity (<40 nm), the oligonucleotide probes hybridize and ligate with two additional connector oligonucleotides to form a continuous circular DNA structure. When a DNA polymerase amplifies these circular structures through rolling-circle amplification with fluorescent nucleotides, each interacting protein pair lights up as a fluorescent dot inside the cell.

The PLA enables the analysis of endogenous protein-protein interactions. A) Primary antibodies bind to two potentially interacting targets. B) These antibodies are recognized by secondary antibodies, which are conjugated to a matched pair of short single-stranded oligonucleotides (PLA probes). C) If the two targets interact and are in close proximity (<40 nm), the oligonucleotide probes hybridize and ligate with two additional connector oligonucleotides to form a continuous circular DNA structure. D) DNA polymerase amplifies these circular structures through rolling-circle amplification with fluorescent nucleotides (Figure modified after https://www.sigmaaldrich.com/technical-documents/protocols/biology/how-pla-works.html.

Experimentally, PLA staining was performed with the Duolink® In Situ Orange PLA Kit Mouse/Rabbit together with the Duolink® In Situ PLA® Probes and Detection Reagents from Sigma-Aldrich. Cells were seeded in 35 mm glass bottom dishes (MatTek) and fixed with 4% Roti®-Histofix (Carl Roth) for 20 min at room temperature prior to PLA staining. Following three washing steps with PBS, the cells were permeabilized and unspecific binding sites were blocked for 30 min at 37 °C with blocking buffer containing TritonX-100 and normal goat serum. Afterwards, the cells were incubated at 4 °C overnight with two primary antibodies derived from rabbit (anti-Survivin, NB500-201, Novus Biologicals) and mouse (anti-Histone H3, ab195277, abcam or anti-Crm1, sc-74454 Santa Cruz), which were diluted in antibody dilution buffer. Duolink® In Situ PLA probes Anti-Rabbit PLUS and Anti-Mouse MINUS were added after washing three times with PBS and incubated for 1 h at 37°C. Following three additional washing steps, the ligation solution was incubated on the cells for 30 minutes at 37°C. After another three washing steps, the amplification reagents were added and incubated for 100 minutes at 37°C. Cells were then stained with 10 μg/ml Hoechst 33342 and HCS CellMask™ Deep Red Stain (Thermo Scientific) in a dilution of 1:5000 in PBS for 20 min at RT in the dark before they were stored at 4 °C in 0.1% (w/v) sodium azide /PBS until they were microscopically analyzed. Confocal fluorescence microscopy images were taken with the scanning microscope TCS SP8 (Leica Microsystems) equipped with four lasers (Argon: 458/476/488/496/514 nm; DPSS: 561 nm; Helium Neon: 633 nm; UV Diode: 405 nm), two
PMT confocal imaging detectors and one sensitive imaging hybrid detector. The samples were imaged with an HCX PL APO CS 63.0 x / 1.20 water objective or an HCX PL APO 63 x / 1.4–0.6 oil objective. The microscope was operated with the Leica Application Suite X (LAS X) software (Leica Microsystems). Subsequently, maximum projection images of z-stacks were analyzed with Cell Profiler. The outlines of the nuclei were defined based on Hoechst 33342 staining (primary objects) and the outlines of the entire cells were encircled based on Cell Mask staining and defined as secondary objects. PLA foci within the cells were then detected and assigned to the respective parental cells.
9. SRV100 biosensor assay

The SRV100 biosensor assay developed by the group of Jose A. Rodríguez was used to analyze the export activity of Crm1 in the presence or absence of potential Survivin-Crm1 inhibitors in a cellular context. The biosensor (SRV100) is composed of a shortened version of Survivin (1–100), which contains the NES responsible for the interaction with the export receptor Crm1 and in addition two nuclear localization signals (NLS) and a 3 x FLAG-tag. The NLS sequences ensure a nuclear localization of the biosensor in case it is not actively exported by Crm1. If no inhibitor is added, Crm1 exports the biosensor into the cytoplasm. If an inhibitor is able to hinder Survivin from interacting with Crm1, the biosensor remains in the nucleus.

293T cells were seeded in 8 well μ-slides (ibidi) 24 h before they were transfected with 300 ng of the plasmids SRV100 (biosensor) and Crm1-GFP. 4 h after transfection 10 µM LNES were added to the culture medium. 24 h after transfection, cells were fixed with 4% Roti®-Histofix (Carl Roth) for 20 min at room temperature prior to immunostaining. Following three washing steps with PBS, the cells were permeabilized and unspecific binding sites were blocked for 30 min at 37 °C with blocking buffer containing TritonX-100 and normal goat serum. Afterwards, the cells were incubated at 4 °C over night with an anti-Flag antibody (Sigma Aldrich, F3165) diluted in antibody dilution buffer followed by three washing steps with PBS and a 1 h incubation with an anti-mouse AF568 secondary antibody (Invitrogen, A11004) at RT. Cells were additionally stained with 10 µg/ml Hoechst 33342 and HCS CellMask™ Deep Red Stain (Thermo Scientific) in a dilution of 1:5000 in PBS for 20 min at RT in the dark before they were stored at 4 °C in 0.1% (w/v) sodium azide/PBS until they were microscopically analyzed.

Biosensor plasmids used in this assay were kindly provided by the group of Jose A. Rodríguez, University of the Basque Country, Spain.

10. Proliferation assay

The CellTiter 96® AQueous One Cell Proliferation Assay (Promega) was used to observe cell proliferation after treatment with different ligand concentrations. The assay determines the number of viable cells per well by using the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) that is bioreduced by NADPH or NADH in living cells into a colored formazan product. Cells were seeded into a black 96-Well glass bottom dish (Corning) and incubated with different ligand concentrations for 72 h (triplicates for each condition). The assay was performed according to the manufacturer’s protocol by adding 20 µl of CellTiter 96® AQueous One Solution Reagent directly into each culture well and incubating for 4 h. Afterwards, the absorption was measured at 490 nm with a GloMax®-Multi plate reader (Promega).
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