Electronic Supplementary Information (ESI)

Amino acid recognition by fine tuning the association constants: tailored naphthalimides in pillar[5]arene-based indicator displacement assays

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1. Materials and methods
2. Synthesis of G1-G3
3. Absorption and fluorescence spectra of G1·WP5, G2·WP5, and G3·WP5 systems
4. Photographs of G1·WP5, G2·WP5, and G3·WP5 systems
5. Job’s plots of G1·WP5, G2·WP5, and G3·WP5 systems
6. Association constant and quantum yield determination
7. Anchor volume calculations
8. Fluorescence indicator displacement
9. NMR studies
10. pH-dependence
11. References
1. Materials and methods

Solvents, reagents and starting materials were obtained from commercial supplier and used without further purification. WP5 and 4-(2-bromoethylamino)-naphthalimide were prepared as described before (see Refs. 50 and 47 in the main paper). All the spectroscopic experiments were carried out at 25°C. The UV–vis absorption spectra were recorded on an Agilent 8453 diode array spectrometer. The fluorescence spectra were measured on an Edinburgh Instruments FLSP 920 fluorescence spectrometer. The $^1$H NMR spectra were taken on a Bruker Avance DRX-500 or DRX-300 spectrometer with chemical shifts reported in ppm (TMS in the case of CDCl$_3$ and the residual DMSO in the case of DMSO-d$_6$ was used as internal standard). The exact mass measurements were performed using a Q-TOF Premier mass spectrometer (Waters Corporation, 34 Maple St, Milford, MA, USA) using electrospray ionization in positive mode.

2. Synthesis of G1-G3

Overview

![Figure S1 Syntheses of the guests G1-G3](image-url)
Synthesis of G1 [1]

The putrescine-conjugated G1 was prepared as previously described with some minor modifications. Namely, 500 mg 4-bromo-9-propyl-1,8-naphthalimide (1) [2] was refluxed with 5.0 g putrescine in 3 ml 2-methoxyethanol for 20 hours. After cooling, the reaction mixture was added to 75 ml water and extracted with dichloromethane 3 times. The organic phase was washed with water and brine, dried on MgSO$_4$ and the solvent was evaporated to yield an amorphous solid. The residue was crystallized overnight in water, filtered, washed with water several times and dried in vacuo to yield 235 mg (46%) yellow powder.

$^1$H NMR (300 MHz, CDCl$_3$) δ 8.55 (d, $J = 7.3$ Hz, 1H), 8.43 (d, $J = 8.4$ Hz, 1H), 8.20 (d, $J = 7.9$ Hz, 1H), 7.55 (t, $J = 7.9$ Hz, 1H), 6.65 (d, $J = 8.5$ Hz, 1H), 6.59 (s, 1H), 4.13 (t, $J = 7.5$ Hz, 2H), 3.40 (dt, $J = 10.5$, 5.0 Hz, 2H), 2.86 (t, $J = 6.5$ Hz, 2H), 1.93 (p, $J = 6.8$ Hz, 2H), 1.85 – 1.62 (m, 4H), 1.52 (s, 3H), 1.01 (t, $J = 7.4$ Hz, 3H).

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 164.77, 164.19, 150.09, 134.55, 130.94, 129.84, 126.71, 124.31, 122.88, 120.40, 109.54, 103.95, 43.71, 41.64, 41.39, 30.59, 26.05, 21.49, 11.60.

HRMS: calculated 326.1869 for [MH]$^+$ $C_{19}H_{24}N_3O_2$; found: 326.1870
Figure S2 $^1$H-NMR spectrum of G1

Figure S3 $^{13}$C-NMR spectrum of G1
Synthesis of G2

The imidazolium-anchored G2 was prepared by refluxing 4-(2-bromoethylamino)-naphthalimide (2, 200 mg, 0.67 mmol) with 1-methylimidazole (82 mg, 1.0 mmol) in 6 ml acetonitrile for 20 hours. The mixture was cooled to room temperature, filtered and washed three times with acetonitrile to yield 106 mg (37%) of pure product.

$^1$H NMR (300 MHz, DMSO-d6) δ 9.16 (s, 1H), 8.65 (d, J = 8.5 Hz, 1H), 8.44 (d, J = 7.2 Hz, 1H), 8.27 (d, J = 8.5 Hz, 1H), 7.86 (t, J = 5.7 Hz, 2H), 7.79 (s, 1H), 7.71 (m, 2H), 6.90 (d, J = 8.6 Hz, 1H), 4.52 (t, J = 5.2 Hz, 2H), 4.03 – 3.92 (m, 2H), 3.88 (d, J = 5.8 Hz, 5H), 3.83 (s, 3H), 1.62 (h, J = 7.4 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H).

$^{13}$C NMR (75 MHz, DMSO) δ 164.18, 163.43, 150.40, 137.47, 134.44, 131.25, 129.78, 129.12, 125.14, 123.99, 123.34, 122.42, 120.88, 109.31, 104.65, 47.98, 43.06, 41.27, 40.88, 40.60, 40.32, 40.04, 39.77, 39.49, 39.21, 36.25, 21.47, 11.88.

HRMS: calculated 363.182 for [M]$^+$ C$_{21}$H$_{23}$N$_4$O$_2$; found: 363.1820

Figure S4 $^1$H-NMR spectrum of G2
Synthesis of G3

The trimethylammonium-anchored G3 was prepared by refluxing 4-(2-bromoethylamino)naphthalimide (2, 150 mg, 0.42 mmol) with trimethylamine (4.2 M ethanolic solution, 1 ml, 4.2 mmol) in 5 ml acetonitrile for 20 hours. The mixture was cooled to room temperature, filtered and washed three times with acetonitrile to yield 80 mg (57%) of pure product.

$^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.73 (d, $J = 8.5$ Hz, 1H), 8.45 (d, $J = 7.3$ Hz, 1H), 8.30 (d, $J = 8.4$ Hz, 1H), 7.92 (s, 1H), 7.73 (t, $J = 7.9$ Hz, 1H), 6.93 (d, $J = 8.5$ Hz, 1H), 3.97 (t, $J = 6.4$ Hz, 2H), 3.71 (t, $J = 6.4$ Hz, 2H), 3.23 (s, 9H), 1.62 (h, $J = 7.4$, 6.9 Hz, 2H), 0.90 (t, $J = 7.4$ Hz, 3H).

$^{13}$C NMR (126 MHz, DMSO) δ 163.64, 162.92, 149.41, 133.87, 130.78, 129.18, 128.21, 124.83, 122.03, 120.35, 109.08, 104.45, 62.65, 52.80, 40.78, 40.04, 39.96, 39.87, 39.80, 39.70, 39.63, 39.54, 39.46, 39.37, 39.20, 39.03, 36.90, 20.93, 11.35.

HRMS: calculated 340.2025 for [M]+ $C_{20}H_{26}N_{43}O_2$; found: 340.2018
Figure S6 $^1$H-NMR spectrum of G3

Figure S7 $^{13}$C-NMR spectrum of G3
3. Absorption and fluorescence spectra of G1-WP5, G2-WP5, and G3-WP5 systems

Figure S8 Variation of the absorption (left) and fluorescence (right) spectra of G1 (1.0 μM) in HEPES buffer (pH 7.4) upon the addition of WP5. Absorption spectra: 5 cm path length, fluorescence spectra: λ<sub>ex</sub> = 450 nm, 0 to 50 equivalent WP5; the inset shows the fluorescence emission vs. host concentration at 550 nm.

Figure S9 Variation of the absorption (left) and fluorescence (right) spectra of G2 (1.0 μM) in HEPES buffer (pH 7.4) upon the addition of WP5. Absorption spectra: 5 cm path length, fluorescence spectra: λ<sub>ex</sub> = 399 nm, 0 to 128 equivalent WP5; the inset shows the fluorescence emission vs. host concentration at 550 nm.
Figure S10 Variation of the absorption (left) and fluorescence (right) spectra of G3 (1.0 µM) in HEPES buffer (pH 7.4) upon the addition of WP5. Absorption spectra: 5 cm path length, fluorescence spectra: λ<sub>ex</sub> = 433 nm, 0 to 1280 equivalent WP5; the inset shows the fluorescence emission vs. host concentration at 550 nm.
4. Photographs of G1·WP5, G2·WP5, and G3·WP5 systems

Figure S11 Different degree of complexation. Photographs of the indicators (15 μM) containing increasing amount (from left to right: 0, 1, 10 and 100 equivalents) of WP5 in HEPES buffer (pH 7.4) under a handheld UV-lamp (365 nm). (a) G1 (b) G2 (c) G3
5. Job’s plots of G1∙WP5, G2∙WP5, and G3∙WP5 systems

**Figure S12** Job’s plot of the fluorescence emission of the G1∙WP5 system at 550 nm, in HEPES buffer (pH 7.4). The sum of the concentrations is 1 µM. The dashed lines represent 1:1 and 2:1 (guest-host) stoichiometries.

**Figure S13** Job’s plot of the fluorescence emission of the G2∙WP5 system at 550 nm, in HEPES buffer (pH 7.4). The sum of the concentrations is 1 µM. The dashed lines represent 1:1 and 2:1 (guest-host) stoichiometries.
Figure S14 Job's plot of the fluorescence emission of the G3-WP5 system at 550 nm, in HEPES buffer (pH 7.4). The sum of the concentrations is 1 µM. The dashed lines represent 1:1 and 2:1 (guest-host) stoichiometries.

6. Association constant and quantum yield determination

The association constants have been obtained from the emission spectra of the dyes and from the spectra of samples with the same concentration of G and different concentrations of WP5. The emission spectra have been corrected by the spectral sensitivity of the instrument. For the evaluation of the spectra the scheme of a two-step association

\[ G + WP5 \leftrightarrow G \cdot WP5 \] (equilibrium constant \( K_{a1} \))

and

\[ G + G \cdot WP5 \leftrightarrow G_2 \cdot WP5 \] (equilibrium constant \( K_{a2} \))

was applied.

The equilibrium constants were obtained by a least-square fitting, in which the concentrations of the three fluorescent species, G, G-WP5 and G2-WP5, were expressed from the equations

\[ K_{a1} = \frac{c_G^{GWP5}}{c_j \cdot c_j^{WP5}} \]

\[ K_{a2} = \frac{c_j^{G2WP5}}{c_j \cdot c_j^{GWP5}} \]

and from the molar balance for G

\[ c_j^{GWP5} + c_j^{GWP5} + c_j^G = c_0^G, \]
and substituted into the sum of squared residuals

\[ S_{tot}^{em} = \sum_{i=1}^{n} \sum_{j=1}^{m} \left( l_{i,j} - \phi_i^G \cdot c_j^G - \phi_i^{G_{WP5}} \cdot c_j^{G_{WP5}} - \phi_i^{G_{2_{WP5}}} \cdot c_j^{G_{2_{WP5}}} \right)^2 \]

where \( l_{i,j} \) denotes the corrected emission intensity of the \( j \)-the sample at the \( i \)-the wavelength, \( \phi_i^G \), \( \phi_i^{G_{WP5}} \) and \( \phi_i^{G_{2_{WP5}}} \) are the relative emission intensities of the respective forms.

Performing a minimization of \( S_{tot}^{em} \) using \( \phi_i^{G_{WP5}} \), \( \phi_i^{G_{2_{WP5}}} \), \( K_{a1} \) and \( K_{a2} \) as fitting parameters yielded the values of the stepwise association constants.

<table>
<thead>
<tr>
<th>Table S1. Stepwise association constants for the complexes ( G_{-WP5} ) and ( G_{2-WP5} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log K_{a1} )</td>
</tr>
<tr>
<td>( G1\text{-WP5} )</td>
</tr>
<tr>
<td>( G1_{2}\text{-WP5} )</td>
</tr>
<tr>
<td>( G2\text{-WP5} )</td>
</tr>
<tr>
<td>( G3\text{-WP5} )</td>
</tr>
</tbody>
</table>

**Determination of fluorescence quantum yields of indicator molecules and inclusion complexes**

The fluorescence quantum yields of the indicator and complex were determined using coumarin 153 [3] (\( \Phi = 0.62 \)) and Rhodamine 6G (\( \Phi = 0.94 \)) [4] as reference. The optical density were lower than 0.05 to avoid inner filter effects. The quantum yields have been calculated using the equation

\[ \Phi = \Phi_R \cdot \frac{I}{I_R} \cdot \frac{OD_R}{OD} \cdot \frac{n^2}{n_R^2} \]

where \( \Phi \) is the quantum yield, \( I \) is the integrated fluorescence intensity, \( OD \) is the optical density, and \( n \) is the refractive index (R refers to the reference dye).
7. Anchor volume calculations

The anchor volumes were computed with Density functional theory (DFT) calculations using the Gaussian 09 program package.[1] For geometry optimizations we employed the PBE0 (called PBE1PBE [2-4]) functional with the 6-311G standard basis set augmented with polarization and diffuse functions - 6-311++G**-. The volume values below are defined as the volumes inside a contour of 0.001 electrons/Bohr$^3$ density. [1]

<table>
<thead>
<tr>
<th>Ion</th>
<th>cm$^3$/mol</th>
<th>Å$^3$/molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylammonium ion</td>
<td>46.06</td>
<td>76.77</td>
</tr>
<tr>
<td>$N,N'$-ethyl-methyl imidazolium ion</td>
<td>86.25</td>
<td>143.75</td>
</tr>
<tr>
<td>$N,N$-ethyl-trimethylammonium ion</td>
<td>102.58</td>
<td>170.97</td>
</tr>
</tbody>
</table>

8. Fluorescence indicator displacement, LOD calculations

![Fluorescence titration curves of G1-WP5](image)

*Figure S15. Fluorescence titration curves of G1-WP5 (1.0 μM indicator, 15 equivalent host, 95% complexation) with cadaverine (left, 0 to 0.65 mM) and arginine (right, 0 to 1.28 mM)*
Figure S16 Fluorescence titration curves of \textbf{G2\_WP5} (1.0 \(\mu\)M indicator, 15 equivalent host, 80\% complexation) with cadaverine (top left, 0 to 0.65 mM), arginine (top right, 0 to 0.65 mM) and lysine (bottom, 0 to 1.28 mM)

**Limit of detection calculations**

*Calculations shown on the example of G1\_WP5 and arginine*

To the first 7 points of the titration curve at 550 nm intensity was fitted a linear equation:

\[
y = 6.73 \cdot 10^4 x + 26.0
\]

\[
S = 6.73 \cdot 10^4
\]

\[
\delta = \sqrt{\frac{\sum (F - \bar{F})^2}{(N-1)}} = 0.301 \quad N=20, \; K=3
\]

\[
LOD = \frac{K \times \delta}{S} = 1.34 \cdot 10^{-5} \text{M}
\]
Table S2 LOD values for the FID assays

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD for G1-WP5</th>
<th>LOD for G2-WP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cad</td>
<td>$9.44 \times 10^{-7}$ M</td>
<td>$1.51 \times 10^{-7}$ M</td>
</tr>
<tr>
<td>Arg</td>
<td>$1.34 \times 10^{-5}$ M</td>
<td>$3.27 \times 10^{-6}$ M</td>
</tr>
<tr>
<td>Lys</td>
<td></td>
<td>$2.61 \times 10^{-5}$ M</td>
</tr>
</tbody>
</table>
9. NMR studies

Figure S17 $^1$H-NMR spectra (500 MHz, 25°C) of (a) cadaverine (b) cadaverine+$G_1$ (c) $G_1$ in D$_2$O. The concentration of the analyte was 30 mM, the indicator was 3 mM.

Figure S18 $^1$H-NMR spectra (500 MHz, 25°C) of (a) arginine (b) arginine+$G_1$ (c) $G_1$ in D$_2$O. The concentration of the analyte was 30 mM, the indicator was 3 mM.
Figure S19 $^1$H-NMR spectra (500 MHz, 25°C) of (a) cadaverine (b) cadaverine$+\text{G2}$ (c) G2 in D2O. The concentration of the analyte was 30 mM, the indicator was 3 mM.

Figure S20 $^1$H-NMR spectra (500 MHz, 25°C) of (a) arginine (b) arginine$+\text{G2}$ (c) G2 in D2O. The concentration of the analyte was 30 mM, the indicator was 3 mM.
Figure S21 $^1$H-NMR spectra (500 MHz, 25°C) of (a) $G_1 \cdot WP_5$ (b) cadaverine+$G_1 \cdot WP_5$ (c) $G_1$ in D2O. The concentration of the analyte was 30 mM, the indicator and $WP_5$ was 3 mM. In this case, complete indicator displacement was observed.

Figure S22 $^1$H-NMR spectra (500 MHz, 25°C) of (a) $G_1 \cdot WP_5$ (b) arginine+$G_1 \cdot WP_5$ (c) $G_1$ in D2O. The concentration of the analyte was 30 mM, the indicator and $WP_5$ was 3 mM. The poor quality of spectrum (b) is attributed to precipitation.
Figure S23 $^1$H-NMR spectra (500 MHz, 25°C) of (a) $\text{G2} \cdot \text{WP5}$ (b) cadaverine+$\text{G2} \cdot \text{WP5}$ (c) $\text{G2}$ in D2O. The concentration of the analyte was 30 mM, the indicator and $\text{WP5}$ was 3 mM. In this case, complete indicator displacement was observed.

Figure S24 $^1$H-NMR spectra (500 MHz, 25°C) of (a) $\text{G2} \cdot \text{WP5}$ (b) arginine+$\text{G2} \cdot \text{WP5}$ (c) $\text{G2}$ in D2O. The concentration of the analyte was 30 mM, the indicator and $\text{WP5}$ was 3 mM. In this case, partial indicator displacement was observed and the resulting spectrum represents an average signal set.
10. pH-dependence

Association constants

Table S3 pH-dependence of the association constants

<table>
<thead>
<tr>
<th>Complex</th>
<th>pH 6.8</th>
<th>pH 7.4</th>
<th>pH 8.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1-WP5</td>
<td>5.96</td>
<td>6.09</td>
<td>5.54</td>
</tr>
<tr>
<td>G2-WP5</td>
<td>5.34</td>
<td>5.45</td>
<td>5.34</td>
</tr>
</tbody>
</table>

Note: log K values measured in the pH range afforded by HEPES buffers

pH-dependence of indicator displacement for G2-WP5

![Fluorescence regeneration values](image)

Figure S25 Fluorescence regeneration values (F/F\textsubscript{unbound}) at 550 nm of cadaverine, arginine and lysine of G2-WP5 system at different pH values in HEPES. The concentration of the indicator was 1 µM and 15 equivalent of host was added. The analytes were added in 3 mM concentration. The basic amino acids show decreasing values upon increasing the pH due to the pH-sensitivity of their complexation with WP5. The different values compared to Fig. 5 in the main text is due to the different concentration values.
11. References


