Fluorescence of Tryptophan in Designed Hairpin and Trp-cage Miniproteins: Measurements of Fluorescence Yields and Calculations by Quantum Mechanical Molecular Dynamics Simulations

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SUPPORTING INFORMATION

1. Molecular Dynamics Parameters

The center of mass of the protein ($\mathbf{X_M}$) was constrained to a point ($\mathbf{X_0}$) by subjecting each protein atom (i) to a force $\mathbf{F_{M,i}} = -k_M(\mathbf{X_M} - \mathbf{X_0})m_i/M$, where m_i is the atomic mass, M is the total mass of the protein, and $k_M = 10^4$ kcal·mol^{-1}·Å^{-2}. To hold the water molecules in a sphere with radius R around $\mathbf{X_0}$, force $\mathbf{F_{R,j}} = -k_R\mathbf{D_j}$ was applied to each water O atom j for which $D_j > R$, where $D_j = |\,\mathbf{D_j}\,|$, $\mathbf{D_j} = \mathbf{X_j} - \mathbf{X_0}$, $\mathbf{X_j}$ is the position of j, and $k_R = 25$ kcal·mol^{-1}·Å^{-2}. The total angular momentum of the water ($\mathbf{L_W}$) was held to zero by subjecting each water atom j (including hydrogens) to a force $\mathbf{F_{L,j}} = -k_L m_j \mathbf{L_W} \mathbf{X} \mathbf{v_j}$, where $\mathbf{v_j}$ is the velocity of atom j, $k_L = 5\times10^{-5}$ kcal·mol^{-1}·(Da·Å^2·fs^{-1})$^{-2}$.

Tables S1 and S2 give the parameters used for stretching and bending of the bonds of 3-methylindole and tryptophan in the ground and excited singlet (^1L_a) states.

Table S1. Bond-stretching Parameters.

Bond [a]		Ground State		^1L_a	
atom *i*	atom *j*	b_0 [b]	f_b [c]	b_0 [b]	f_b [c]
CG	CD1	1.344	474.7	1.428	375.9
CG	CD2	1.451	356.6	1.400	403.8
CD1	NE1	1.377	430.5	1.354	455.1
NE1	CE2	1.391	413.9	1.416	391.2
CE2	CZ2	1.400	403.8	1.377	430.5
CE2	CD2	1.382	424.4	1.414	389.2
CZ2	CH2	1.399	404.9	1.502	325.9
CH2	CZ3	1.386	419.7	1.364	447.1
CZ3	CE3	1.397	407.1	1.423	380.5
CE3	CD2	1.412	391.2	1.462	348.6
NE1	HE	0.980 [d]	434.0 [d]	1.000	434.0
CZ2	HZ1	1.000 [d]	340.0 [d]	0.980	340.0
CE3	HE2	1.000 [d]	340.0 [d]	0.980	340.0

[a] Atom designations are shown in the structure to the right. Indole atom 3 is CG.
[b] Ideal bond length (Å).
[c] Force constant (kcal·mol^{-1}·Å^{-2}).
[d] Enzymix default value for bonds of this type.

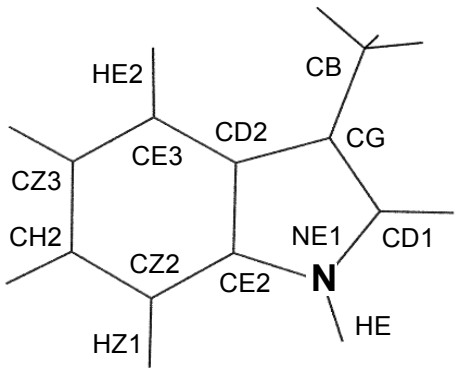

Table S2. Bond-bending parameters.

Angle [a]			Ground	^1L_a
atom *i*	atom *j*	atom *k*	θ_0 [b]	θ_0 [b]
CB	CG	CD1	128.0	130.0
CB	CG	CD2	126.5	127.3
CD1	CG	CD2	105.5	102.6
CG	CD1	NE1	111.5	111.0
CD1	NE1	CE2	107.4	111.8
NE1	CE2	CZ2	129.0	132.7
NE1	CE2	CD2	107.8	104.7
CZ2	CE2	CD2	123.2	123.3
CG	CD2	CE3	131.2	131.3
CG	CD2	CE2	107.7	111.6
CE2	CD2	CE3	121.1	117.2
CZ3	CE3	CD2	114.6	122.8
CH2	CZ3	CE3	124.8	120.5
CZ2	CH2	CZ3	119.7	119.6
CE2	CZ2	CH2	116.4	118.8

[a] Atoms are designated as in Table S1.
[b] Ideal bond angle (degrees). A force constant of 85 kcal·mol^{-1}·radian^{-2} was used for all these angles.

2. Contributions of Induced Dipoles to Reorganization Energies

Figure S1 shows distributions of the reorganization energies associated with induced dipoles, as calculated by eq. (22) for excitations of the hairpin peptide with Trp in position 1 and protonated His in position 8 (Ac-WVTIpGKHIFTG-NH_2). The left-most curve (*red*, peak at 30 cm^{-1} off scale vertically) is the reorganization energy of the first excited singlet π–π* state of Trp1. The other curves are the reorganization energies for electron transfer from the indole ring of Trp1 to the protonated imidazole ring of His8 (*black*) and amides *a*1 (*magenta*), *a*2 (*blue*), *a*9 (*cyan*), and *a*8 (*green*), where the number following "*a*" indicates the residue that provides the amine group for the amide. The histograms include data from twenty 0.5-ns trajectories in the lowest excited singlet π–π* state, and are normalized to constant area. Trajectories were propagated in steps of 1 fs, and $\Delta E^{g}_{k,n}$ and $\Delta E^{e}_{k,n}$ were evaluated every 500 steps. The quantum charges were scaled by a factor of 0.8 before they were used in the MD forcefield (see Figure S4 below).

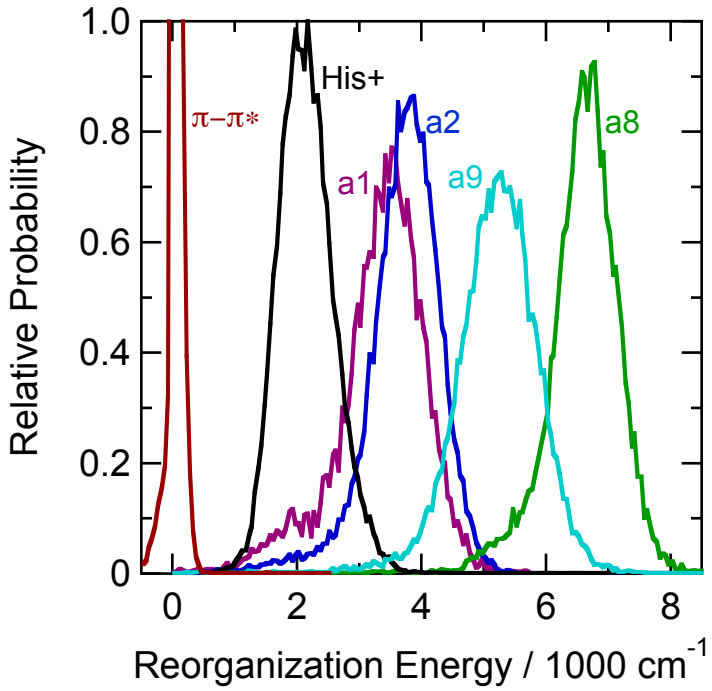

Figure S1.

3. Calculated Properties of Liquid Water

Dipole moment. Figure S2 shows the distribution of dipole moments calculated for liquid water as described in the text. The mean dipole moment ($|\mu_{total}|$) was 2.95 debye (D), including 2.34 D from the permanent charges of the atoms ($|\mu_{perm}|$). As noted in the text, the permanent charges in the ENZYMIX force field implicitly incorporate some polarization because they are parameterized for molecules in solution. The atomic polarizabilities of H and O were adjusted to bring the mean $|\mu_{total}|$ into accord with the experimental value (2.95 ± 0.2 D)[1] and *ab initio* MD calculations by Silvestrelli and Parrinello.[2] The latter calculations gave the same mean but a broader distribution, possibly because the computational model was smaller (12 MD configurations of 64 water molecules), and thus presumably more sensitive to edge effects. The distribution of $|\mu_{total}|$ shown in Fig. S1 is fit well by a Gaussian, $\exp\left[-(|\mu_{total}|-\mu_0)^2/2\sigma^2\right]$ with $\mu_0 = 2.94$ and $\sigma = 0.194$ D. Calculations by Bernardo et al.,[3] Burnham and Xantheas,[4] Ren and Ponder[5] and Jensen *et al.*[6] have given mean dipole moments between 2.55 and 2.81 D.

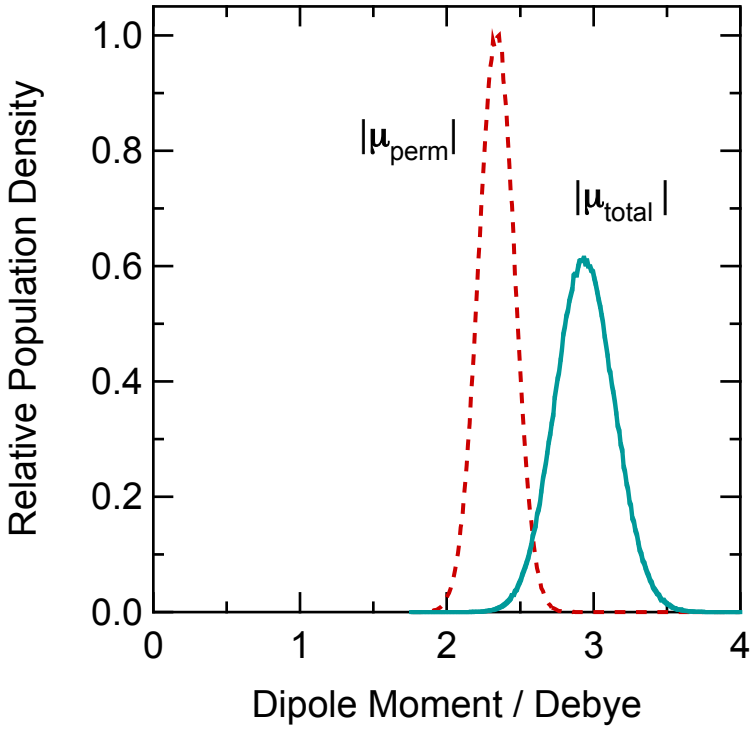

Figure S2.

Radial distribution functions. To evaluate the O-O and O-H radial distribution functions (g_{OO} and g_{OH}), we averaged over the distributions around each of the ~261 water molecules with an O atom within 12 Å of the center of each ensemble, not including the molecule that was held at the center. The O-O distribution (g_{oo}, Figure S3*A*) peaks at 2.73 Å, in reasonable accord with the distances between 2.73 and 2.83 Å obtained in current analyses of X-ray and neutron diffraction data.[7-9] The peaks at 1.74 and 3.23 Å in g_{OH} (Figure S3*B*) match the peaks in the distributions derived from diffraction data to within ±0.05 Å.

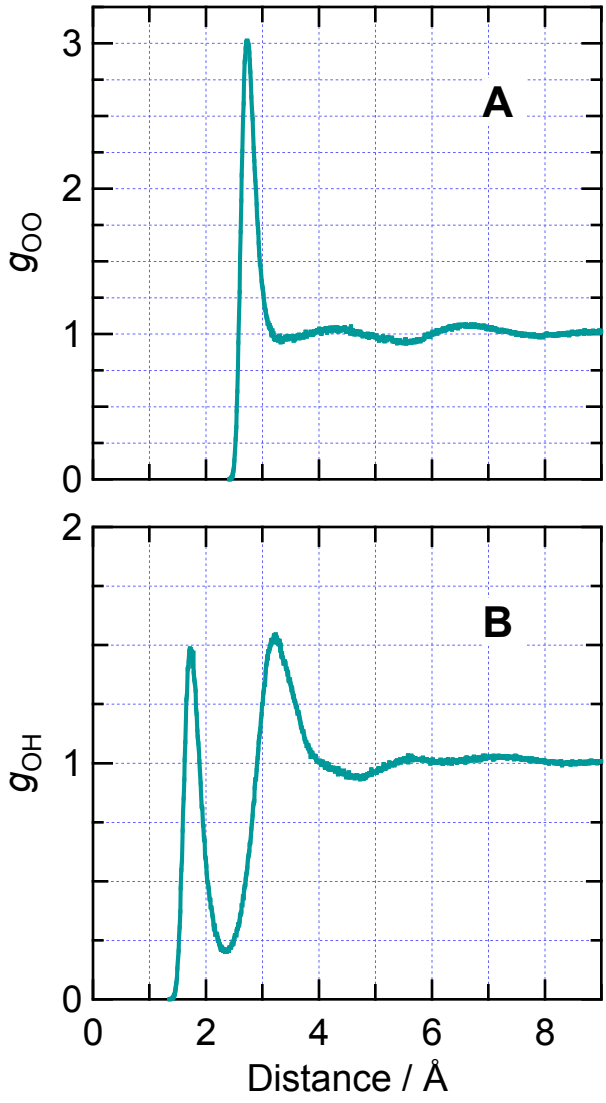

Figure S3.

Bond length and angle. The O-H bond length, H-O-H bond angle and molecular dipole moment were averaged over the 1000 ensembles saved during the simulation and over the ~746 molecules with an O atom within 17 Å of the center of each ensemble (again not counting the molecule at the center), leaving the shell between 17 and 21 Å to buffer edge effects. The mean bond length was 1.015 Å, in agreement with the experimental value of 1.01 Å reported by Soper & Benmore,[7] but somewhat greater than the 0.97 Å measured by Ichikawa *et al*.[10] and Silvestrelli and Parrinello's[2] *ab initio*-MD value of 0.991 Å. The mean bond angle of 106.0° matched both the measured angle[10] and the value obtained by *ab initio* MD calculations.[2]

Diffusion coefficient. The translational diffusion coefficient of water was obtained by identifying the water molecules within 8 Å of the center of each ensemble (skipping the central molecule again), and tracking their positions at 0.5-ps intervals for the 25-ps period represented by the next 50 ensembles. The mean-square displacement (MSD) at each delay time was averaged over all the possible ensembles (950 ensembles for the maximum delay of 25 ps, 999 for 0.5 ps). The period of 25 ps was short enough so that none of the molecules that were tracked approached the surface of the 21-Å sphere. Neglecting the expected nonlinearity at times below 0.5 ps, the MSD increased linearly with a slope (s) of 1.477 ± 0.001 $Å^2$/ps, giving a diffusion coefficient (s/6) of 0.246 Å2/ps or 2.46×10^{-5} cm^2/s. The experimental value for water at 300 K is $2.30 \times 10^{-5} cm^2/s$.[11,12]

4. Calculated Fluorescence Properties of 3-Methylindole in Water

Figure S4 shows calculated emission peak (*A* and *C*) and full width at half-maximum amplitude (FWHM, *B* and *D*) of 3-methylindole (3MI), when the atomic charges or classical potentials were scaled by variable factors. Simulations of 3MI in a 16-Å sphere of water were propagated as described in the text. In panels *A* and *C*, the quantum charges of the 3MI atoms were multiplied by the factor indicated on the abscissa before they were used in the MD forcefield. In panels *B* and *D*, the classical potentials from the solvent atoms were multiplied by the indicated factor before they were added to the diagonal terms of the Fock Hamiltonian.

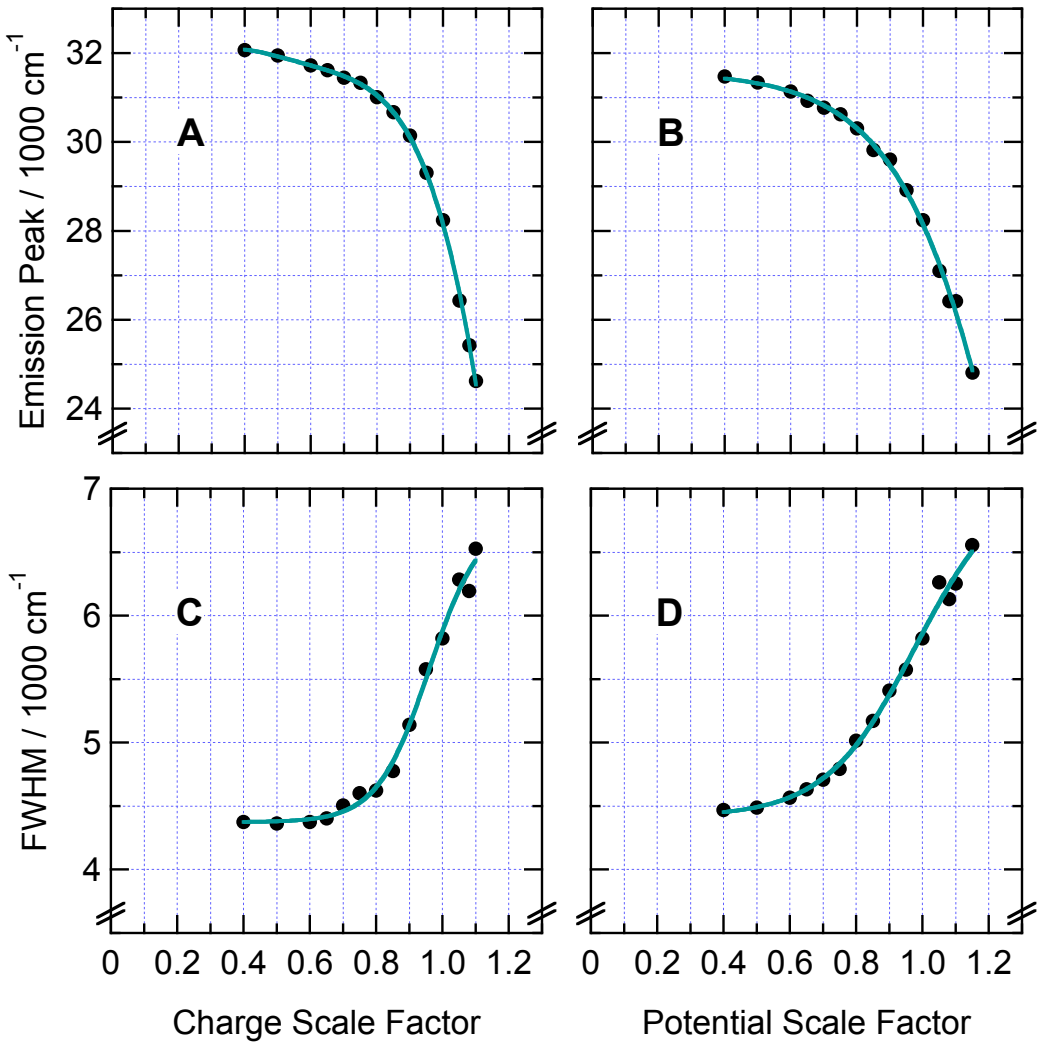

Figure S4.

5. NMR Structures of the His8 Hairpin Peptide

An ensemble of 17 of the 20 accepted NMR structures of the β-hairpin peptide Ac-WVTIpGKHIFTG-NH_2 is shown in Figure S5. The Mean global root-mean-square deviations (RMSDs) of the backbone atoms and all heavy atoms were 0.69 ± 0.34 and 1.25 ± 0.45 Å, respectively. Table S3 gives the atomic coordinates of the structure we used for MD simulations and for generating initial structures of other peptides of the hairpin series.

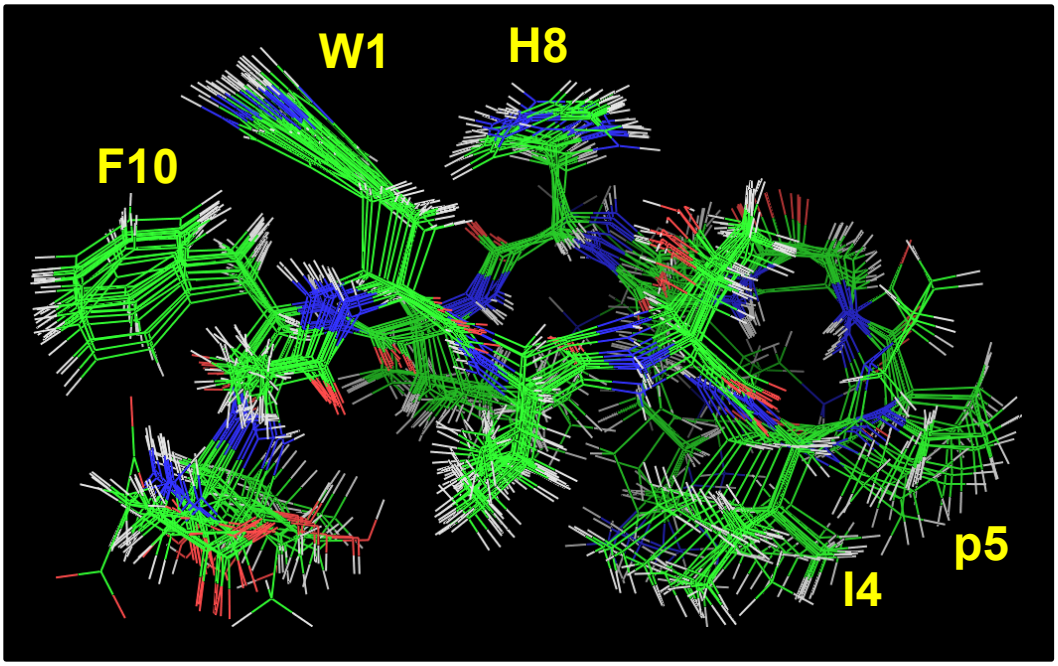

Figure S5.

Table S3.

```
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REMARK Accepted structure 1 of 20 structures.
REMARK Molecular dynamics scheme : torsion; torsion; cartesian; minimize.
REMARK High temperature dynamics :
REMARK      temp: 50000; steps: 1000; time(ps): 15.
REMARK 1st cooling stage :
REMARK      temp: 50000->0; steps: 1000; time(ps): 15; temp step: 250.
REMARK 2nd cooling stage :
REMARK      temp: 2000->0; steps: 3000; time(ps): 15; temp step: 25.
REMARK Total of 2000 steps of minimization.
REMARK VDW scale factors 0.1; 0.1->1; 1->4; 1.
REMARK 154 NOEs in 1 class(es) with scale factors of 150; 150; 150; 75.
REMARK      averaging function: sum.
REMARK 0 3-bond j-couplings.
REMARK 0 1-bond j-couplings.
REMARK 0 carbon chemical shifts.
REMARK 0 proton chemical shifts.
REMARK 0 diffusion anisotropy restraints.
REMARK 0 susceptability anisotropy restraints.
REMARK 0 dihedral restraints.
REMARK 0 planarity restraints.
REMARK NCS restraints not used.
REMARK ======================================================
REMARK               bond, angles, improp, vdw(<1.6),  dihed
REMARK violations :     0       1       0         0       6
REMARK RMSD       : 0.0060   0.658   0.491            26.029
REMARK ======================================================
REMARK                noe,   cdih,   coup,   oneb, carb-a, carb-b,
REMARK violations :     0       0       0       0       0   -----
REMARK RMSD       :  0.063   0.000   0.000   0.000   0.000   0.000
REMARK 0.2/2 viol.:     5       0       0
REMARK ======================================================
REMARK               dani,   sani
REMARK violations :     0       0
REMARK RMSD       :  0.000   0.000
REMARK .2/.1 viol.:     0       0
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REMARK ncs     = 0
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ATOM    107  HB2 LYS     7      -1.973  -1.623  -5.004  1.00  0.00
ATOM    108  CG  LYS     7      -1.945  -3.438  -6.111  1.00  0.00
ATOM    109  HG1 LYS     7      -2.932  -3.863  -6.215  1.00  0.00
```

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ATOM    110  HG2 LYS     7      -1.211  -4.230  -6.099  1.00  0.00
ATOM    111  CD  LYS     7      -1.669  -2.531  -7.300  1.00  0.00
ATOM    112  HD1 LYS     7      -0.687  -2.095  -7.187  1.00  0.00
ATOM    113  HD2 LYS     7      -2.411  -1.748  -7.322  1.00  0.00
ATOM    114  CE  LYS     7      -1.722  -3.297  -8.613  1.00  0.00
ATOM    115  HE1 LYS     7      -1.877  -2.594  -9.418  1.00  0.00
ATOM    116  HE2 LYS     7      -2.551  -3.989  -8.578  1.00  0.00
ATOM    117  NZ  LYS     7      -0.467  -4.059  -8.867  1.00  0.00
ATOM    118  HZ1 LYS     7      -0.335  -4.786  -8.135  1.00  0.00
ATOM    119  HZ2 LYS     7       0.351  -3.416  -8.854  1.00  0.00
ATOM    120  HZ3 LYS     7      -0.513  -4.522  -9.797  1.00  0.00
ATOM    121  C   LYS     7      -2.485  -2.648  -2.388  1.00  0.00
ATOM    122  O   LYS     7      -3.001  -1.685  -1.821  1.00  0.00
ATOM    123  N   HIS     8      -1.486  -3.364  -1.878  1.00  0.00
ATOM    124  HN  HIS     8      -1.121  -4.098  -2.407  1.00  0.00
ATOM    125  CA  HIS     8      -0.884  -3.098  -0.586  1.00  0.00
ATOM    126  HA  HIS     8      -1.403  -2.276  -0.116  1.00  0.00
ATOM    127  CB  HIS     8      -0.999  -4.342   0.287  1.00  0.00
ATOM    128  HB1 HIS     8      -0.056  -4.871   0.275  1.00  0.00
ATOM    129  HB2 HIS     8      -1.763  -4.979  -0.108  1.00  0.00
ATOM    130  CG  HIS     8      -1.350  -4.038   1.700  1.00  0.00
ATOM    131  ND1 HIS     8      -2.638  -4.041   2.194  1.00  0.00
ATOM    132  HD1 HIS     8      -3.449  -4.247   1.684  1.00  0.00
ATOM    133  CD2 HIS     8      -0.560  -3.706   2.721  1.00  0.00
ATOM    134  HD2 HIS     8       0.500  -3.608   2.670  1.00  0.00
ATOM    135  CE1 HIS     8      -2.610  -3.715   3.476  1.00  0.00
ATOM    136  HE1 HIS     8      -3.465  -3.627   4.128  1.00  0.00
ATOM    137  NE2 HIS     8      -1.355  -3.508   3.821  1.00  0.00
ATOM    138  HE2 HIS     8      -1.034  -3.393   4.740  1.00  0.00
ATOM    139  C   HIS     8       0.576  -2.714  -0.812  1.00  0.00
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ATOM    142  HN  ILE     9       0.308  -0.915   0.024  1.00  0.00
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ATOM    151 HG21 ILE     9       4.028   0.251  -3.087  1.00  0.00
ATOM    152 HG22 ILE     9       2.538  -0.380  -3.795  1.00  0.00
ATOM    153 HG23 ILE     9       3.520  -1.405  -2.751  1.00  0.00
ATOM    154  CD1 ILE     9      -0.024  -0.347  -2.539  1.00  0.00
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ATOM    163  HA  PHE    10       4.219  -0.086   2.839  1.00  0.00
ATOM    164  CB  PHE    10       5.813  -1.523   2.681  1.00  0.00
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ATOM    165  HB1 PHE    10       6.581  -1.772   1.965  1.00  0.00
ATOM    166  HB2 PHE    10       5.214  -2.404   2.856  1.00  0.00
ATOM    167  CG  PHE    10       6.487  -1.106   3.986  1.00  0.00
ATOM    168  CD1 PHE    10       7.762  -0.531   3.979  1.00  0.00
ATOM    169  HD1 PHE    10       8.283  -0.388   3.043  1.00  0.00
ATOM    170  CD2 PHE    10       5.848  -1.266   5.224  1.00  0.00
ATOM    171  HD2 PHE    10       4.861  -1.702   5.272  1.00  0.00
ATOM    172  CE1 PHE    10       8.370  -0.137   5.159  1.00  0.00
ATOM    173  HE1 PHE    10       9.357   0.307   5.141  1.00  0.00
ATOM    174  CE2 PHE    10       6.467  -0.871   6.397  1.00  0.00
ATOM    175  HE2 PHE    10       5.966  -1.001   7.348  1.00  0.00
ATOM    176  CZ  PHE    10       7.722  -0.309   6.360  1.00  0.00
ATOM    177  HZ  PHE    10       8.197  -0.001   7.272  1.00  0.00
ATOM    178  C   PHE    10       5.781   0.766   1.657  1.00  0.00
ATOM    179  O   PHE    10       6.941   0.603   1.271  1.00  0.00
ATOM    180  N   THR    11       5.191   1.957   1.699  1.00  0.00
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ATOM    182  CA  THR    11       5.890   3.171   1.287  1.00  0.00
ATOM    183  HA  THR    11       6.922   2.909   1.118  1.00  0.00
ATOM    184  CB  THR    11       5.294   3.699  -0.027  1.00  0.00
ATOM    185  HB  THR    11       5.684   4.688  -0.213  1.00  0.00
ATOM    186  OG1 THR    11       3.880   3.790   0.054  1.00  0.00
ATOM    187  HG1 THR    11       3.497   2.909   0.064  1.00  0.00
ATOM    188  CG2 THR    11       5.635   2.840  -1.230  1.00  0.00
ATOM    189 HG21 THR    11       5.627   3.450  -2.121  1.00  0.00
ATOM    190 HG22 THR    11       4.906   2.050  -1.329  1.00  0.00
ATOM    191 HG23 THR    11       6.617   2.409  -1.099  1.00  0.00
ATOM    192  C   THR    11       5.835   4.245   2.377  1.00  0.00
ATOM    193  O   THR    11       5.579   5.418   2.093  1.00  0.00
ATOM    194  N   GLY    12       6.090   3.831   3.624  1.00  0.00
ATOM    195  HN  GLY    12       6.297   2.885   3.780  1.00  0.00
ATOM    196  CA  GLY    12       6.079   4.756   4.751  1.00  0.00
ATOM    197  HA1 GLY    12       6.987   5.341   4.727  1.00  0.00
ATOM    198  HA2 GLY    12       6.060   4.185   5.667  1.00  0.00
ATOM    199  C   GLY    12       4.887   5.700   4.742  1.00  0.00
ATOM    200  OT1 GLY    12       3.738   5.211   4.756  1.00  0.00
ATOM    201  OT2 GLY    12       5.106   6.929   4.720  1.00  0.00
ENDMDL
```

6. RMSD and RMSF Plots from Simulations of Peptides in the Ground State

Panel *A* of Figure S6 shows the root-mean-square deviations (RMSDs) of the Cα carbons from their initial positions during ground-state simulations of the hairpin peptide Ac-WVTIpGKAIFTG-NH_2. Panels *B* and *C* show the same for the analogs with His replacing Ala8 (*B*) or Phe10 (*C*). *D* and *E* show the RMSDs for peptides with Tyr replacing Ala8 or Phe10; *F* and *G*, for peptides with Glu, Gln, Asp or Asn at position 8. The peptides containing His or Tyr were simulated with the side chains of these residues in both their neutral (H or Y, *black curves*) and ionized (H+ or Y–, *blue* and *red*, respectively) forms. The MD trajectories were propagated in 1-fs steps at 300 K, and the RMSD was calculated at 1-ps intervals.

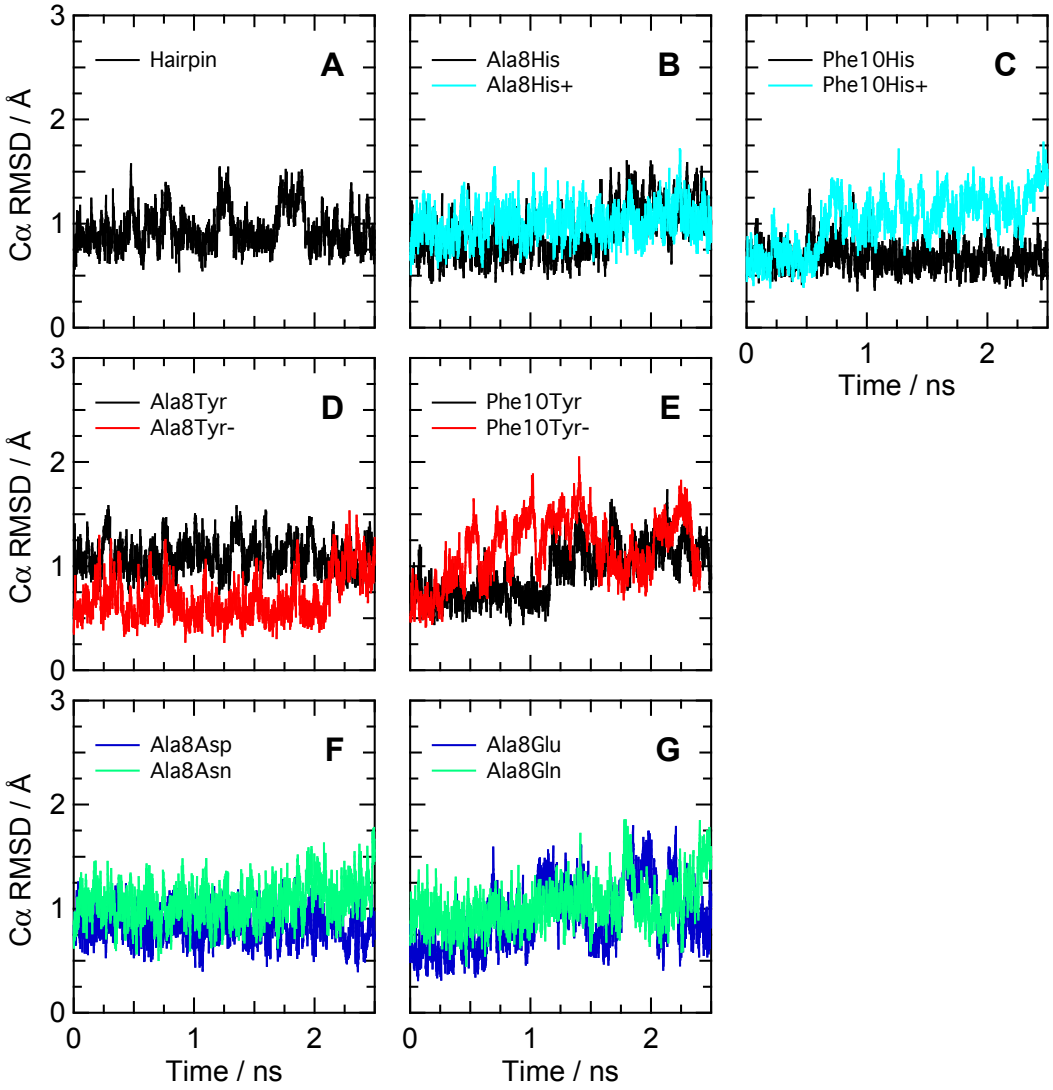

Figure S6.

Panels *A*-*C* of Figure S7 show the Cα RMSDs from ground-state trajectories of the TrpCage16b peptide DAFAQWLADaGPASaRPPPS (*A*, *black curve*), and its variants with Ala replacing Asp9 (*A*, *cyan*), citrulline (Cit) replacing Arg16 (*B*, *black*), Leu replacing Asp9 (*B*, *cyan*), Ala replacing Asp9 and norvaline (Nva) replacing Arg16 (*C*, *black*), or Leu replacing Asp9 and Ile replacing Arg16 (*C*, *cyan*). Panels *D*-*F* show the Cα RMSDs from trajectories of TrpCage10b peptide, DAFAQWLKDGGPASGRPPPS, with norvaline (Nva) replacing Arg16 (*A*, *black curve*), Glu replacing Asp9 (*A*, *cyan*), ΔSer20 (*B*, *black*), ornithing (Orn) replacing Arg16 (*B*, *cyan*), Arg replacing Asp9 and Glu replacing Arg16 (*C*, *black*), or Ala replacing Lys8 (*C*, *cyan*).

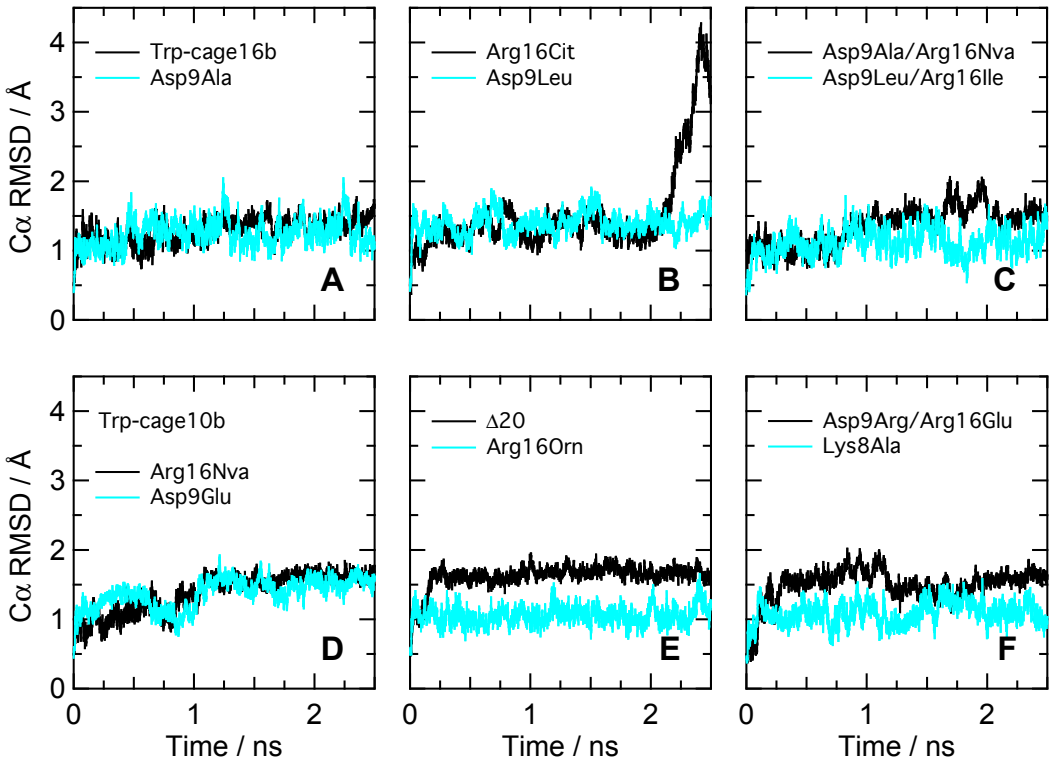

Figure S7.

Figure S8 shows representative plots of the root-mean-square fluctuations (RMSFs) of the Cα carbons about their mean positions in the same simulations as Figures S6 and S7.

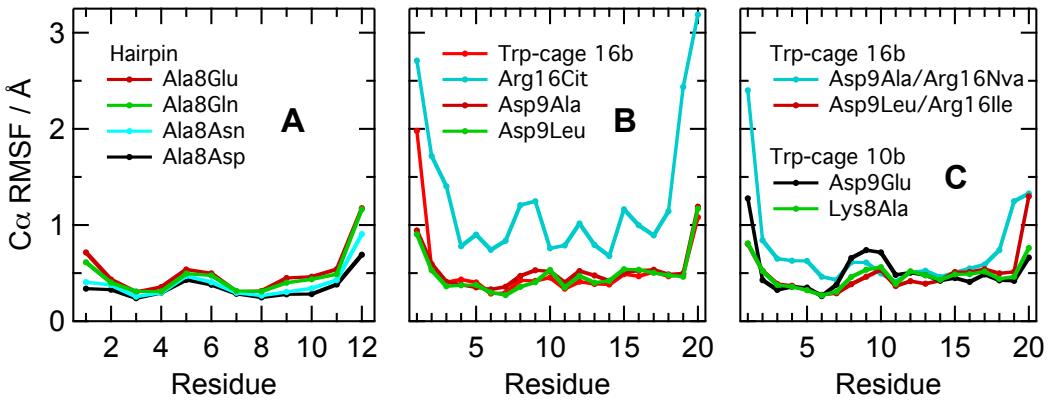

Figure S8.

7. Calculated π–π* and CT Energies of Additional Peptides

Figure S9 shows the calculated energies of the first two excited singlet π–π* states and lowest four CT states of the hairpin peptide with Trp at position 1 and either serine (*A*), cyclohexylalanine (*B*) or ε-N-acetyllysine (*C*) relacing Ala at position 8. The results are similar to those obtained with the Ala8 peptide.

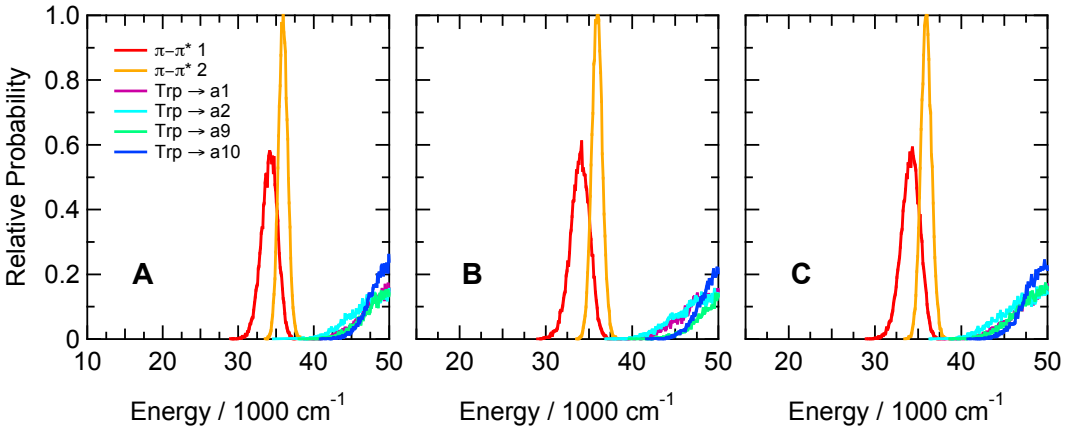

Figure S9.

Figure S10 shows the energy distributions for excited singlet π–π* states and CT states of the Arg16Nva variant of the Trp-cage10b peptide (*A*), and the Asp8Ala and Asp9Leu/Arg16Ile variants of TrpCage16b peptide (*B* and *C*, respectively). The results are similar to those obtained with the other Trp-cage peptides (Figure 8).

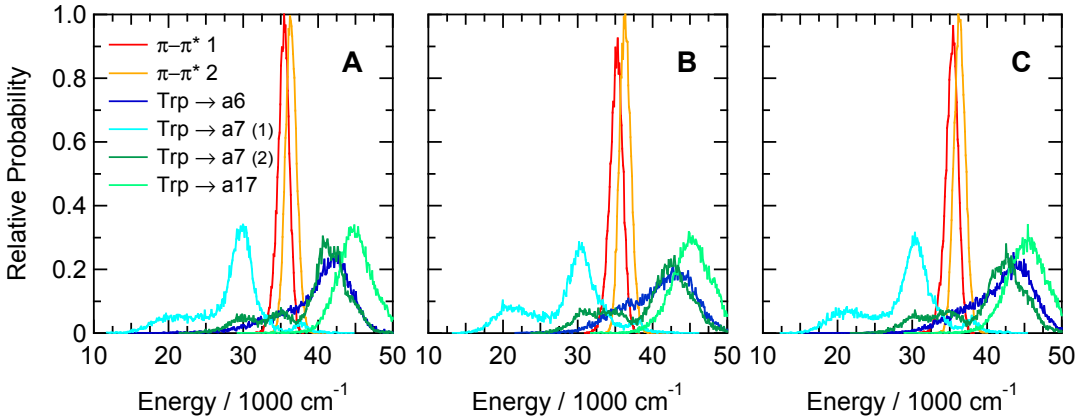

Figure S10.

8. Predicted Fluorescence Yields

Figure S11 shows the correlation of the measured fluorescence yields with the yields predicted by using eq. (24) with $\phi_0 = 0.2475$. The results are for the same 26 peptides used in Figure 10 of the text, and the symbols are the same as in that figure (*circles*, hairpin peptides; *squares*, Trp-cage 16b peptides; and *triangles*, Trp-cage 10b peptides). Other details are given in the legend to Figure 10. The *straight line* is a linear least-squares fit to the data (R^2 = 0.836, Pearson correlation coefficient = 0.914, *y*-intercept = 0.0050 ± 0.0132, slope = 0.9411 ± 0.0851. Note that eq. (24) does not consider the magnitude of the electron-transfer matrix element. Equations (25) – (27) give better overall agreement with experiment, particularly for the peptides with low fluorescence yields (see Figure 10). Plots of $|V|^2 \xi / \left(|V|^2 + \hbar\xi / 2\pi \right)$ and ρ_{FC}, the two fluctuating functions that enter into eq. (25) are shown in Fig. S12. Table S4 gives the detailed results of both treatments, along with the measured fluorescence yields and their errors.

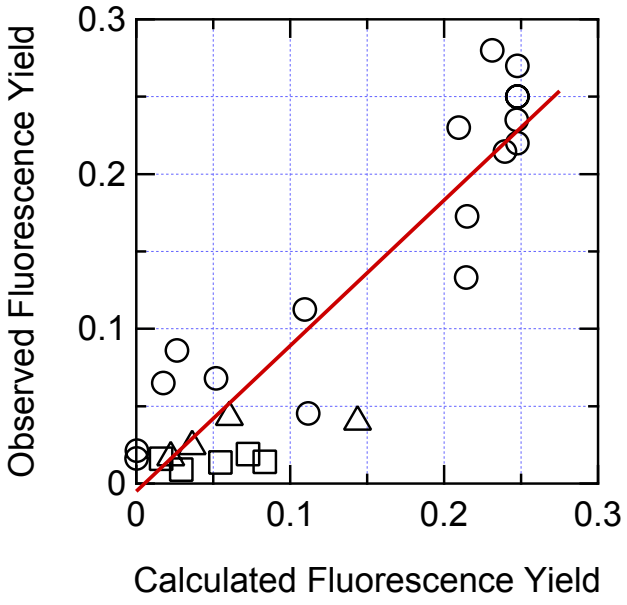

Figure S11.

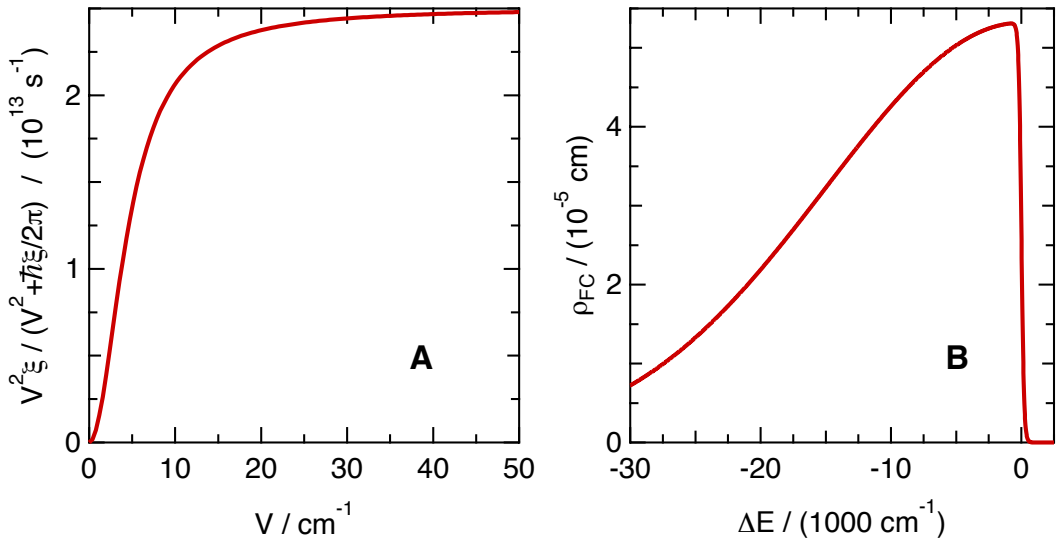

Figure S12.

Table S4

Peptide	Φ_f obs [a]	Φ_f calc [b]	Φ_f calc [c]
Hairpin [d]	0.25 ± 0.02	0.2475	0.2475
Ala8Nak	0.25 ± 0.01	0.2475	0.2475
Ala8Cha	0.27 ± 0.01	0.2475	0.2475
Ala8Ser	0.22 ± 0.01	0.2475	0.2475
Ala8Asn	0.28 ± 0.03	0.2313	0.1817
Ala8Gln	0.23 ± 0.02	0.2096	0.1427
Ala8Asp	0.0649 ± 0.0065	0.0176	0.0240
Ala8Glu	0.1124 ± 0.0113	0.1095	0.0436
Ala8His	0.2351 ± 0.0122	0.2471	0.2461
Ala8His+	0.0860 ± 0.0063	0.0265	0.0332
Ala8Tyr	0.1332 ± 0.0027	0.2144	0.1530
Ala8Tyr-	0.0162 ± 0.0061	0.0003	0.0354
Phe10His	0.2145 ± 0.0091	0.2394	0.2241
Phe10His+	0.0454 ± 0.0054	0.1116	0.0662
Phe10Tyr	0.1726 ± 0.0042	0.2149	0.1514
Phe10Tyr-	0.0212 ± 0.0082	0.0003	0.0536
Trp1Phe/Phe10Trp	0.068 ± 0.005	0.0519	0.0298
Trp-cage 16b [e]	0.009 ± 0.001	0.0293	0.0384
Asp9Leu	0.019 ± 0.002	0.0726	0.0475
Asp9Ala/Arg16Nva	0.014 ± 0.001	0.0835	0.0497
Arg16Cit	0.016 ± 0.003	0.0162	0.0363
Asp9Leu/Arg16Ile	0.014 ± 0.004	0.0547	0.0438
Trp-cage 10b [f]			
Asp9Arg / Arg16Glu	0.040 ± 0.002	0.1438	0.0727
Asp9Glu	0.043 ± 0.005	0.0604	0.0457
ΔSer20	0.024 ± 0.001	0.0363	0.0393
Lys8Ala	0.017 ± 0.003	0.0223	0.0375

[a] Observed fluorescence yield. The values for hairpin peptides with Asp, Glu, His or Tyr at position 8 or 10 are the asymptotes of the titration curves shown in Figure 2 of the main text.

[b] Fluorescence yield calculated using eq. (24) with $\phi_0 = 0.2475$.

[c] Fluorescence yield calculated using eqs. (25) – (27) with the parameter values given in the text.

[d] Hairpin peptide Ac-WVTIpGKAIFTG-NH_2 with substitutions as indicated (Nak = *N*-acetyllysine, Cha = cyclohexylalanine). Ionized forms of His and Tyr are denoted by + and -.

[e] Trp-cage peptide 16b (DAFAQWLADaGPASaRPPPS) with substitutions as indicated (Nva = norvaline).

[f] Trp-cage peptide 10b (DAFAQWLKDGGPASGRPPPS) with substitutions or deletions as indicated.

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11. References for Supplementary Material

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