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The color of cation- π interactions: Subtleties of amine-tryptophan interaction energetics allow for radical-like visible absorbance and fluorescence

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Abstract

Several peptides and a protein with an inter- or intramolecular cation- π interaction between tryptophan (Trp) and an amine cation are shown to absorb and fluoresce in the visible region of the spectrum. Titration of indole with sodium hydroxide or ammonium hydroxide yields an increasing visible fluorescence as well. Visible absorption and multi-peaked fluorescence excitation spectra correlate with experimental absorption spectra and the vibrational modes of calculated absorption spectra for the neutral Trp radical. The radical character of the cation-indole interaction is predicted to stem from the electrostatic dislocation of indole highest occupied molecular orbital (HOMO) charge density towards the cation with a subsequent electronic transition from the HOMO-2 to the HOMO. Because this is a vertical transition, fluorescence is possible. Hydrogen bonding at the indole amine most likely stabilizes the radical-like state. These results provide new spectroscopic tools for the investigation of cation- π interactions in numerous biological systems, among them, proteins and their myriad ligands, and show that one, or at most, two, point mutations with natural amino acids are all that is required to impart visible fluorescence to proteins.

Graphical abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental Section and Supplemental Figures:

S1. Mass spectrometric results for L-Arg-L-Trp-2HCl in 50% acetic acid. S2. Image from MD simulation (30 ns) of L-Arg-L-Trp, showing formation of trimer in water. S3. Absorption spectrum for rHSA in buffer solution. S4. Fluorescence emission spectra for Trp-containing peptides. S5. QM/MD isosurfaces for cation- π interaction between the ammonium cation and the indole residue of Trp. Fluorescence lifetime decay raw data (visible excitation) and analysis for: S6a. Solid state L-Arg-L-Trp, S6b. Solid state L-Arg-L-Trp-2HCl, S6c. Puro A in SDS micelles, S7a. rHSA in buffer. S7b. Fluorescence lifetime decay raw data (UV excitation) and analysis for rHSA in buffer. S8. UVR spectrum (229 nm excitation) of Puro A in 20 mM SDS, 20 mM Tris, pH 7.4. S9. UVR (229 nm) W17 peaks for L-Trp and amine-L-Trp, cation- π -containing molecules. S10. Fluorescence excitation spectra for the titration of aqueous indole with molar equivalents of NaOH.

INTRODUCTION

Aromatic amino acids, such as Trp, participate in a noncovalent interaction known as the cation- π interaction.¹ It has been characterized as primarily an electrostatic interaction involving the aromatic π electrons, and univalent cations, among them cationic amines like arginine (Arg), and cationic amine ligands like acetylcholine.^{2,3} In its simplest configuration, the cation is positioned above a single aromatic ring plane, where electrostatic interaction with π electrons occurs. These cation- π interactions play a role in the function of a broad range of biological systems,⁴ among them, several important large classes of proteins. They are especially common at protein-protein interfaces, where often more than one is present.⁵ Cation- π interactions are also common in a class of peptides and proteins known as Arg and Trp-rich antimicrobial peptides, which act through binding to the anionic outer membrane followed by its disruption.^{6,7} The role of the cation- π interaction in these two protein classes seems to be primarily structural: supramolecular association in the former case and structural disruption in the latter.

In other classes of proteins, the nature of the cation- π interaction suggests that an additional role may come into play. One such class is the ligand-gated ion channels where amine ligands are acetylcholine, dopamine, serotonin, nicotine or gamma-aminobutyric acid^{8–10}. Here, cation- π interaction occurs between the cationic ligand and one or more aromatic residues in an aromatic-lined cavity. This interaction plays a critical role in ion channel function, and these ion channels and receptors are of high interest because they are ‘critical mediators of neurotransmission.’¹⁰ While structural changes contribute to ligand-gating, it is reasonable to consider the localized motion of charge as contributing to the triggering of an ion channel as well.

Theoretical calculations have shown that cation- π interactions in cationic amine-aromatic complexes are dominated by electrostatic interactions,^{3,11} with a significant if not equal contribution from induction (polarization).^{12,13} Kahn et al.¹⁴ have questioned whether biomolecular interactions are accurately simulated when explicit charge transfer terms are absent from molecular mechanics force fields. A DFT/B3LYP study of ammonium cation interaction with indole and other aromatics has shown that the positive charge is delocalized over both the ammonium cation and indole, indicating that charge transfer is involved in the

interaction.¹⁵ An energy decomposition study for ammonium complexes with both benzene and pyrrole shows that this charge transfer is specifically a HOMO→LUMO, π - σ^* donation from the aromatic to the ammonium cation.¹⁶ Other theoretical studies have found that charge transfer contributes significantly to the cation- π interaction energy,^{16–18} and that nonadditive effects need to be considered in their accurate representation.¹⁹ To be sure, inter- and intramolecular charge transfer complexes involving Trp with absorption maxima spanning the 350–510 nm range are well-documented in the literature.^{20–24} However, in most cases, Trp and its acceptor pair are oriented edge-on,^{20–22,24} and do not fit the definition of a cation- π interaction.

Experimental characterization of the cation- π interaction has relied on the molecular level detail provided by x-ray crystallography,^{25,26} NMR spectroscopy⁷ or unnatural amino acid mutagenesis.²⁷ We demonstrate here that both visible absorption and fluorescence spectroscopic techniques can now be used to characterize the cation- π interaction between cationic amines and Trp. Furthermore, we show that peptides and a protein containing the cation- π Trp interaction share electronic and vibrational spectroscopic features with Trp neutral radicals. We hypothesize that the delocalization of Trp HOMO electron density over the cation and the subsequent HOMO-2 →HOMO electronic absorption transition creates a weak, radical-like state.^{28,29}

We show that this technique extends to complexes formed between either Na⁺ or NH₄⁺ and indole. We predict that these spectroscopic techniques can be extended to other cations and other aromatics participating in cation- π interactions, providing researchers with spectroscopic fingerprints for specific noncovalent molecular interactions. We envision a broad impact for these results given the omnipresence of cation- π interactions in proteins and their ligands, especially for protein complexes like ion channels, whose function is to transport charge. Lastly, these results point the way for introducing visible fluorescence into proteins through point mutation with 1 or 2 native amino acids at virtually any selected site with little if any structural perturbation.

RESULTS AND DISCUSSION

The cation- π visible absorption band in Trp-containing dipeptides

The charge transfer band formed between the indole residue of tryptophan and an amine cation first came to our attention because of the intense pink color of solid state L-Arg-L-Trp·2HCl. Other tryptophan-containing dipeptides, such as L-Tyr-L-Trp and L-His-L-Trp, are also pink in color, but of lesser intensity. After eliminating the possibility of artefactual contamination by metal cations³⁰ (See Methods and Materials), we sought information on the nature of the interaction from x-ray crystallographic studies. No structure for L-Arg-L-Trp·2HCl or L-Arg-L-Trp was found, but structures for L-Tyr-L-Trp and L-Trp-L-Ser have been published and are available from the Cambridge Structural Database.³¹ The authors note ‘unusual’ interactions for both dipeptides, where the terminal amine hydrogens form a pair of noncovalent intra- and intermolecular interactions with an aromatic ring. For L-Tyr-L-Trp, this interaction is with two Tyr residues, as shown in Figure 1a, while for L-Trp-L-Ser, the terminal amine interaction is with two Trp residues, as given in Figure 1b. Interatomic distances between the terminal amine hydrogens and ring carbons are given in

Fig. 1. Interestingly, solid L-Trp-L-Ser is also a colored dipeptide. Dougherty and coworkers have extensively studied and characterized these unusual interactions, which are widely known as cation- π interactions^{2,32}.

The unexpected dipeptide color invites spectroscopic investigation. The visible absorption spectra for aqueous solutions of L-Arg-L-Trp, L-Tyr-L-Trp and L-Trp-L-Ser are shown in Figure 2a, c, d, respectively. The visible peaks for all dipeptides are given in Table 1. All visible spectra are resolved into two bands centered at ~ 470 nm and ~ 500 nm and the relative intensity of the peaks vary. Determining the extinction coefficient for these absorption bands is not possible due to the intermolecular nature of the interaction, which diminishes when the dipeptides dissolve.

Nature of the Arg-Trp dipeptide interaction

Because of the intensity of pink color for L-Arg-L-Trp $\cdot$ 2HCl in the solid state and the persistence of visible color for a 50 mg/ml (115 mM) solution in 80% acetic acid reported on the accompanying analytical data sheet,³³ a cation- π interaction between the Arg guanidinium group and the Trp indole was anticipated for lower concentrations.² Mass spectroscopic analysis of the dipeptide, 0.250 mM in 50% acetonitrile/water, 0.1% formic acid, shows the presence of oligomers ranging in size from dimers to octamers (See Supporting Information, Figure S1). Additionally, a 30 ns molecular dynamics (MD) simulation, which began with eight equispaced L-Arg-L-Trp monomers, reveals that such aggregates form and persist throughout the simulation. A characteristic Arg-Trp trimer from the simulation is shown in Figure S2. The simulation image shows that the cation- π interaction is intermolecular with Arg guanidinium groups associating with the face of indole rings. Distances between the nearest neighbor hydrogen on an Arg guanidinium amine group and an indole ring C₄ are 4.04 Å (dipeptide 1-dipeptide 2), and 3.00 Å (dipeptide 2-dipeptide 3), while distances between the nearest neighbor guanidinium hydrogen and C5 are 5.30 Å (dipeptide 1-dipeptide 2), and 3.21 Å (dipeptide 2-dipeptide 3). In the absence of a crystal structure for L-Arg-L-Trp, the mass spectroscopy and MD simulation results support the assignment of a cation- π interaction between the guanidinium group of Arg on one peptide and the π electrons of indole on a second Arg-Trp dipeptide.

To circumvent the loss of intermolecular cation- π interaction and accompanying diminution of the visible absorbance upon dipeptide solid dissolution, intramolecular cation- π interactions were also studied. A thirteen residue fragment (FPVTWRWWKWWKG-NH₂) of the antimicrobial peptide, puromycin A (Puro A), was chosen because the cation- π interaction was of the 'sandwich' type exhibited by L-Trp-L-Ser (Fig. 1b). An NMR structure of this peptide in sodium dodecyl sulfate (SDS) micelles⁷ is shown in Figure 3, where the Arg 6 guanidinium group is sandwiched between the faces of the indoles for Trp 5 and Trp 7. An NMR structure is not available for Puro A in buffer solution, but a CD spectrum has been acquired,³⁴ which shows that structure is imposed on one or more Trp residues. The presence of two Lys residues and three additional Trp residues in Puro A allows for the possibility of a different cation- π interaction under buffer conditions. The visible absorption spectrum for Puro A, 20 mM Tris buffer, pH 7.4, is given in Figure 2b, and shows this to be the case. The absorption peaks are located at 467 nm and 502 nm and

included in Table 1. The Puro A absorption spectrum from 200–600 nm is included as an inset in Fig. 2 to illustrate the relative intensity of the 280 nm absorbance of the five Trp residues. For the peptide in SDS micelles (20 mM SDS addition to the above buffer), absorption peaks are found at 470 and 501 nm (Table 1).

The cation- π interaction in recombinant human serum albumin (rHSA) was examined because this protein contains a single tryptophan in cation- π interaction with the Lys 199 ϵ -amino group, as illustrated in Figure 4. The adjacent Arg 218, with a more distant amide and guanidinium group, is also shown. The rHSA UV-vis absorption spectrum is given in Figure S3 where the 18 Tyr residues dominate the UV absorption at 280 nm. Nevertheless, absorption peaks at 467 nm and 504 nm are still apparent (Table 1).

The visible absorption of HSA has also been measured by pulsed laser-induced optoacoustic spectroscopy, and found to have a maximum at 532 nm.³⁰ Use of this laser-based technique was found necessary because the weak absorbance could not be detected with the conventional configuration of a UV-vis spectrometer. The inability to detect an absorption band was attributed to light scattering by the macromolecule in solution. Here, glycerol has been added to the buffer for rHSA, resulting in a solution refractive index that more closely matches that of the protein, and subsequent recording of the absorption spectrum using a conventional cuvette was possible. Given the evidence: a) success of the laser-based technique³⁰, b) success in detecting the weak, visible absorption band with a light-focusing, fiber optic-based cuvette for other samples, and c) strong fluorescence excitation and emission spectra (discussed below) obtained when samples are excited with the intense light of a xenon arc lamp, we conclude that the absorption transition is enhanced (pumped) by focused, higher intensity visible light. This is analogous to the use of intense UV light (ca. 355 nm) for the creation of Trp radical states.

Concomitantly, visible absorption bands of similar wavelength and intensity have been reported for such UV-excited and radiolysis-generated indole and tryptophan neutral radicals^{35–39}. Peaks for many of these have been included in Table 2.

Briefly, pulsed radiolysis of L-Trp at pH 8–10 yields a single peak at 510 nm with an OD~0.013³⁵ while the neutral radical of indole yields peaks at ~500 nm and ~540 nm⁴⁰.

The 280 nm photolyzed neutral Trp radicals, Az48W $\cdot$ from the native azurin protein, and ReAz108W $\cdot$, from the rhenium-labeled, mutant azurin construct, exhibit dual-peaked absorption bands of 486 nm and 516 nm for the former, and 510 nm and 537 nm, for the latter radical (Table 2)³⁸. The significant wavelength difference between the absorption bands for these two neutral Trp radicals has been explained as resulting from the sensitivity of the radical absorption frequency to local protein environments. The intensity ratio of the two well-resolved peaks for Az48W $\cdot$ is similar to that observed here for several dipeptides while band position for the latter group is blue-shifted an additional ~12–19 nm (Compare with Table 1). Calculated absorption peaks for *P. aeruginosa* Az48W neutral radical are located at 464 nm and 494 nm (Table 2),²⁸ while those for the Cyt C peroxidase Trp 51 neutral radical are calculated as 475 nm and 511 nm.²⁹ Both of these are in excellent agreement with the peaks found here for amine-Trp cation- π interactions.

Extinction coefficient for the visible absorption bands

Calculated molar extinction coefficients for the visible absorption bands of Puro A and rHSA are listed in Table 1. The extinction coefficients for Puro A represent a cation- π interaction where an Arg guanidinium group is sandwiched between two Trp indole residues, as shown in Fig. 3. The value calculated for the shorter wavelength peak is 1696 (M-cm)^{-1} while that for the longer wavelength peak is 2060 (M-cm)^{-1} . These values were calculated relative to the molar extinction coefficient for the 280 nm band (5690 (M-cm)^{-1})⁴² for the five Trps in Puro A. The corresponding extinction coefficients for rHSA, where the cation- π interaction involves a single Trp indole and a Lys ϵ -amino group, are 655 (M-cm)^{-1} and 867 (M-cm)^{-1} . These were calibrated against the 280 nm absorbance to which 18 Tyr and the single Trp contribute. A molar extinction coefficient of $48 \pm 8 \text{ (M-cm)}^{-1}$ was obtained for the laser-induced optoacoustic spectrum of the cation- π interaction in HSA.³⁰ Wide variation in molar extinction coefficient for the neutral Trp radical has also been discussed.³⁸ Lower limits of $\sim 950 \text{ (M-cm)}^{-1}$ at 486 nm and $\sim 1250 \text{ (M-cm)}^{-1}$ at 516 nm are placed on the extinction coefficient for the Az48W neutral radical whereas an extinction coefficient of $2300 \pm 150 \text{ (M-cm)}^{-1}$ has been reported for the neutral Trp radical, pH 7–10³⁵. Greater accuracy due to the inclusion of copper(II) as an internal standard for the Az48W neutral radical, difference in Trp environment, or radical decay are discussed as factors that could account for the two-fold disparity in measured extinction coefficients.³⁸ The variation in the type, number and orientation of the molecular species participating in the cation- π interaction could all contribute to the extinction coefficient difference obtained for Puro A versus rHSA. The observed difference in color intensity for the Trp dipeptide solids discussed above supports this idea.

Interestingly, pulsed radiolysis of indole in N_2O -saturated aqueous solution at pH 9.22, showed that in the presence of Br_2^- radical, the indole neutral radical visible band intensity increased by a factor of 2.3⁴⁰. Analogous results were found for the indole radical cation at pH 3.5. A range of 500 to 2000 (M-cm)^{-1} has been reported for the extinction coefficient of charge transfer bands of indole with phthalimides and phthalyl derivatives of Trp.²⁰ Therefore, a single value for the molar extinction coefficient for all types of cation- π interactions as well as for Trp radicals seems unlikely. In fact, it may be possible to use the extinction coefficient as a metric for identifying a specific type of cation- π interaction or other cases where Trp is not in a closed shell state.

The cation- π interaction is fluorescent

Due to the orientation of the amine cation above the indole ring plane, π electron density is shifted towards the cation,¹⁵ creating a charge deficit in the Trp highest occupied molecular orbital (HOMO). Charge population analysis of several NH_4^+ -aromatic interactions shows that the positive charge is distributed over both molecular species, implying charge transfer, with the greatest effect for the NH_4^+ -indole complex.¹⁵ Visible light-activated excitation from lower lying π orbitals creates a vertical transition, providing for fluorescence between ground state orbitals⁴³ instead of the more typical fluorescent transition from an excited state to the ground state.

As proof of principle and to eliminate interference from amino acid backbone groups, titration of equimolar quantities of NH_4OH with aqueous indole were monitored by visible fluorescence emission, as shown in Figure 5a. The visible emission, maximum at 512 nm (Table 1), saturated at 8 equivalents with a pK of 10.92 or at 5 molar equivalents of NH_4OH . A parallel experiment replacing NH_4OH with NaOH was performed because computed gas phase binding energies show that Na^+ binding to indole is 6.7 kcal/mol greater than that of NH_4^+ .^{44,45} Both cations have been shown to prefer orientation over the benzyl portion of indole.^{11,15,45} Fluorescence emission results for the titration of NaOH with indole are given in Fig. 5b. The emission maximum is 498 nm (Table 1) with a maximum intensity 3.8 times greater than that found for the NH_4OH titration, and which saturated at 5 equivalents of NaOH . The pK calculated for the Na^+ -indole complex from the emission data is 12.28 or at the point of addition of three NaOH molar equivalents. A consideration is the effect of the OH^- counter ion in stabilizing the complex. Since the pK_a for the aqueous indole amine is 16.9 while that for the indole in Trp is 16.82,⁴⁶ the indole amine is not deprotonated in these base titrations, and so a stabilization of the Na^+ -indole- OH^- complex through hydrogen bonding at the indole amine is possible.

The Na^+ -indole- OH^- complex is represented in Scheme 1. The interaction energy between the cation and aromatic quadrupole moment has been shown to have a $1/r^n$ dependence, r being the distance between the ion and quadrupole and $n < 2$.⁴⁷ The ground state shift of electron density from the indole HOMO towards Na^+ and the subsequent visible absorption transition of $\text{HOMO}-2 \rightarrow \text{HOMO}$ is expected to be stabilized by hydrogen bond formation at the indole amine hydrogen with the hydroxyl anion. Involvement in a strong hydrogen bond at the indole amine for the Trp neutral radical has been predicted.^{48,49}

Fluorescence emission spectra in the visible region for solid state L-Tyr-L-Trp and aqueous L-Arg-L-Trp·2HCl are given in Figure S4a. Their emission maximum is found at 529 nm and 503 nm, respectively. Interestingly, even UV excitation of L-Arg-L-Trp→2HCl in the solid state reveals the emission at 515 nm. The fluorescence difference spectrum, L-Arg-L-Trp·2HCl–L-Trp (aq), given in Fig. S4b, reveals the 515 nm peak and an higher energy peak at 450 nm, attributable to a $\text{HOMO}-3 \rightarrow \text{HOMO}$ transition (See section on Cation- π Vibrationally resolved Excitation Spectra, below).²⁹

The emission maximum for all other aforementioned Trp dipeptides, in both the solid state and aqueous solution, are included in Table 1.

The emission maxima for both aqueous and solid state Trp dipeptides span a large wavelength range: from 501 nm to 522 nm for the aqueous phase, and 505 nm to 529 nm for the solid state. The emission maxima for L-Trp-L-Ser appear at 505 nm (solid state) and 506 nm (aqueous). The L-His-L-Trp emission maxima are found at 510 nm (solid state) and 501 nm (aqueous). Since the cation- π interaction for the L-Trp-L-Ser dipeptide is known to involve the N-terminal amine³¹, it could be inferred that this is also the nature of the interaction for L-His-L-Trp. The emission maxima for both phases of L-Tyr-L-Trp appear at longer wavelengths: 522 nm for the aqueous phase and 529 nm for the solid phase. Here, the terminal amine cation interaction is with the phenyl ring of Tyr and not the Trp indole.³¹ Thus cation- π interactions with Tyr are also spectroscopically active. The relatively longer

emission wavelength for this dipeptide may be due solely to the involvement of two Tyr residues in the interaction rather than Trp, or possibly both Tyr and Trp residues participate in the cation- π interaction. We make this suggestion based on the emission maximum for Puro A in micelles. NMR (Fig. 3) shows that an Arg guanidinium group is 'sandwiched' by two Trp indoles in Puro A in micelles, and the fluorescence emission maximum is at 520 nm for this species, 14 nm red shifted relative to the emission maximum in the solid state.

The emission maxima for both L-Arg-L-Trp and L-Arg-L-Trp-2HCl in the solid state are found at 515–19 nm while that for aqueous L-Arg-L-Trp-2HCl is at 503 nm. The emission maximum for aqueous L-Arg-L-Trp is located at 519 nm. These maxima are all reported in Table 1.

The emission maximum for rHSA in 25 mM Tris, 25 mM MES, pH 7.0, 10% (v) glycerol is at 515 nm, as given in Table 1.

Fluorescence emission spectra for Puro A in the solid state and in 20 mM SDS, 20 mM Tris buffer, pH 7.4, are given in Figure S4c. Here, the emission maximum for the peptide in SDS micelles is at a longer wavelength (520 nm) than that for the solid phase (506 nm). In buffer, the Puro A emission maximum is at 515 nm (Table 1). The NMR structure for micelle-associated Puro A (Fig. 3) reveals the double cation- π interaction of Arg 6 with Trp 5 and Trp 7, which accounts for the associated visible fluorescence. Lys 9 and Lys 12 are also in the vicinity of Trp 10, and may have some cation- π interaction.⁷ The CD spectrum for Puro A in SDS micelles indicates some α -helical secondary structure, and a negative band at 228 nm, characteristic of Trp-Trp excitonic interaction, is also present.³⁴ The Trp 5/Trp 7 pair involved in the cation- π interaction would seem to be the most likely candidates for such interaction given their proximity and near coplanarity.

An NMR structure for Puro A in 20 mM buffer is not available, but the CD results indicate that it is a random coil.⁷ However, in buffer the peptide is not totally devoid of structure because Trp-Trp excitonic interaction is indicated in the CD spectrum as well with a negative peak at 225 nm and a corresponding weak, positive peak at 234 nm. Given that Puro A contains 5 Trp residues, it is not possible to say which Trp residues contribute to the excitonic CD feature in the solution phase, but given that visible fluorescence is also obtained and that the CD for the peptide in micelles also shows this Trp excitonic feature, the possibility exists that any two of the five Trps form a pair that are involved in a cation- π interaction with Arg 6, either of the two Lys residues or the N- or C-terminal amine. This interaction is possible even though Puro A is not in a micelle.

Fluorescence Quantum Yields for rHSA upon UV and Visible Excitation

Because rHSA contains a single Trp, which partakes in a cation- π interaction, measurement of fluorescence quantum yield (QY) for both the UV (280 nm) and visible (467 nm) transitions is possible. QY results are given in Table 1. The QY for UV excitation is quite low, 0.012. Such a low QY has been tabulated for several proteins⁵¹, and has been linked to deprotonation at the indole amine, and diminished, uneven electron density across the indole ring with greatest negative charge concentrated at the indole amine.⁵²

Charge distribution maps for an energy minimization of the interaction between an amine cation, NH_4^+ , with tryptophan, are shown in Figure S5. This interaction is presented as a simplified model for the cation- π interactions studied here. In the absence of the cation (Fig. S5, top), charge density over the indole ring plane is fairly evenly distributed and negatively charged, as shown by the yellow color (negative) over the ring surface. The interaction between NH_4^+ and tryptophan in the zwitterionic state shows that when the NH_4^+ is centered over either the pyrrole (Fig. S5, middle) or phenyl (Fig. S5, bottom) ring, electron density is diminished on the ring isosurface immediately below the NH_4^+ position but not on the portion of the indole ring where the NH_4^+ is absent. When the NH_4^+ is positioned over the phenyl ring, negative charge is concentrated on the pyrrole ring with the higher charge density on the indole amine and along the $\text{C}_2\text{---C}_3$ pyrrole bond, as indicated by the red coloration. These simulation results support the idea that juxtaposed cations can distort the π electron density over the indole ring, and that diminished, uneven π electron density over indole ring is associated with reduced quantum yield.⁵²

In parallel, the QY upon visible excitation for rHSA is also very low, 0.007. Low QY for indoles when excited in the UV has been related to deprotonation of the indole amine,⁵² and so it can be reasoned that the indole amine's hydrogen of rHSA is affected. The implication of hydrogen bonding or deprotonation at the indole amine invites further comparison with the Trp neutral radical as both theoretical⁴⁸ and EPR⁴⁹ studies indicate indole amine deprotonation or involvement in a strong hydrogen bond for this species.

Quantum mechanics/molecular mechanics models of the Az48W neutral radical have been constructed to reproduce its spectral features including UV-vis absorbance and fluorescence.²⁸ Mulliken D_0 state charge distribution maps for the Trp neutral radicals, Az48W and ReAz108W, are comparable to the electron density isosurface in Fig. S5, bottom, where the ammonium cation is positioned over the phenyl ring and higher negative charge density is found on the pyrrole ring. Upon visible light excitation, the calculated D_2 state Mulliken maps for both azurin neutral radicals show an increase in negative charge on the pyrrole ring, and consequently, stronger hydrogen bonding at the indole amine.²⁸ This is similar to findings for our own model system.⁵²

Fluorescence emission at ~540 nm for Az48W $\cdot$ and at ~560 nm for ReAz108W has also been predicted by these calculations, although it has not been observed. It is noteworthy that a high background has been incidentally reported in the visible (blue-green) resonance Raman spectroscopy of Az48W and ReAz108W neutral radicals, and attributed to scattering and/or fluorescence.³⁸

These parallels in ground state skewed charge distribution over the indole ring for a cation- π interaction and Az48W and ReAz108W neutral radicals together with their respective observed (501–529 nm, Table 1) and predicted (~540/~560 nm) fluorescence emission further support a charge transfer model for the cation- π interaction, and reinforce a photophysical relationship between the cation- π interaction and the Trp neutral radical state.

Visible and UV Fluorescence Lifetime Measurements

Solid state fluorescence lifetime measurements for the *visible* emission were carried out on both L-Arg-L-Trp and L-Arg-L-Trp·2HCl. These results are given in Table 1. Corresponding decays and decay analyses are included in Figure S6a (L-Arg-L-Trp) and Figure S6b (L-Arg-L-Trp·2HCl). For L-Arg-L-Trp, the dominant lifetime component is 2.8 ns long, while a 2.5 ns lifetime component is slightly under 50% for L-Arg-L-Trp·2HCl. Both lifetime measurements returned a subnanosecond component of considerable amplitude: the 0.68 ns component is, in fact, 52% in amplitude for L-Arg-L-Trp·2HCl. A minor lifetime component (<10%) of 8–9 ns. was found for both L-Arg-L-Trp species, and the resultant weighted average lifetimes were similar: 1.78 ns (L-Arg-L-Trp·2HCl) and 2.44 ns (L-Arg-L-Trp). Interestingly, a subnanosecond lifetime of 0.90 (98%) dominates the *UV* fluorescence lifetime results for L-Lys-L-Trp deprotonated at the indole amine, and a minor but long lifetime of 8.8 ns (1.6%) results in an average lifetime of only 1.0 ns.⁵² Average fluorescence lifetimes excited in the UV for single Trp proteins where the indole amine is hydrogen bound are similarly short (1–2 ns).^{53,54} These short lifetimes are consistent with the association of depleted electron density over the pyrrole ring as discussed above.

The fluorescence lifetime decay (visible excitation) for the π -cation- π ‘sandwich’ motif in Puro A in SDS micelles was also measured, and the lifetimes with their amplitudes are given in Table 1 while the results are shown in Figure S6c. Here, a 4.2 ns component dominates (70%) the decay with a 2.2 ns minor component. Unlike the lifetime decay analysis for the L-Arg-L-Trp dipeptides, no subnanosecond lifetime is found. The resultant weighted average lifetime is 3.6 ns.

The fluorescence lifetime decay (visible excitation) for rHSA in 25 mM Tris, 25 mM MES, pH 7.0, 10% (v) glycerol (Figure S7a), yielded a 4.4 ns component of 54% amplitude (Table 1), which is close to the dominant lifetime for Puro A (4.2 ns). As for the L-Arg-L-Trp dipeptides, significantly longer (12 ns) and shorter (1.1 ns) lifetimes are found with considerable amplitude (21–25%). The visible weighted average lifetime is 5.2 ns.

As for the rHSA quantum yields, the presence of a single tryptophan allows for concurrent measurement of the UV and visible lifetimes for the cation- π interaction. The lifetimes upon UV excitation and their amplitudes, given in Table 1 and Figure S7b, parallel those obtained for the visible lifetime measurement: an identical, dominant lifetime of 4.4 ns flanked by significantly longer (7.8 ns) and shorter (1.5 ns) lifetimes of roughly equal amplitude (19–23%). The UV weighted average lifetime (4.6 ns) is 11% shorter than that for the visible. As discussed above, the very low quantum yield upon UV excitation for rHSA is comparable to values found for L-Lys-L-Trp at pH 13⁵² and for other Trp dipeptides and proteins^{51,55}, where the indole amine is deprotonated or hydrogen bound. However, the UV fluorescence lifetime results for deprotonated L-Lys-L-Trp are very different from those for rHSA. A subnanosecond lifetime of 0.90 (98%) dominates, and a minor but long lifetime of 8.8 ns (1.6%), as mentioned above, contributes to an average lifetime of only 1.0 ns for L-Lys-L-Trp⁵². This difference in lifetime upon UV excitation components suggests another means of spectroscopically distinguish between the cation- π interaction and hydrogen bonding at the indole amine.

The Trp closed shell W17 mode frequency shows hydrogen bonding at the indole amine

For closed shell Trp, the W17 mode is a mixed mode of the ν_{12} mode of benzene and NH motion.⁵⁶ It has been shown that when the indole amine hydrogen participates in a hydrogen bond, *i.e.*, is a hydrogen bond donor, the W17 mode shifts to lower frequency with increased hydrogen bond strength.⁵⁷ The reported frequencies are 883–871 cm^{-1} for nil-to-strong hydrogen bond donation in model Trp compounds.⁵⁷ The UV resonance Raman (229 nm excitation) spectrum for closed shell Trps in Puro A in SDS micelles is shown in Figure S8, and W17 results for L-Trp and other Trps involved in cation- π interactions, as discussed here, are reported in Figure S9 and Table 4. The W17 Raman shifts for L-Arg-L-Trp 2HCl, Puro A, rHSA and the indole-NaOH complex range from 876 to 879 cm^{-1} , moderate values which are consistent with hydrogen bonding to water.⁵⁷ The W17 Raman shift for the closed shell Trp UV resonance Raman spectrum (230 nm excitation) of radical forming Az108W is 880 cm^{-1} , shifting to 875 cm^{-1} in the neutral radical.⁵⁸ A W17 Raman shift of 878 cm^{-1} (229 nm excitation) has been reported for the closed shell His37-Trp41 cation- π interaction at pH 4 in the M2 ion channel of the influenza A virus,⁵⁹ and was also attributed to hydrogen bonding with water. Involvement in a strong hydrogen bond at the indole amine for the Trp neutral radical has been predicted^{48,49} and is supported by the 5 cm^{-1} downshift in the W17 Raman frequency reported for Az108W upon radical formation. The closed shell Trp W17 Raman shifts reported here for several cation- π interactions indicate hydrogen bonding at the indole amine at least as strong as that observed for closed shell Az108W.

Structure in Cation- π Excitation Spectra and the Vibrational Modes of the Trp 191 Neutral Radical

The fluorescence excitation spectra for the indole complex with NaOH and the Puro A peptide, given in Figure 6, show peaks suggestive of underlying vibrational modes. The appearance of vibrational resolution in the excitation spectra is related to the nature of the excitation transition, which we attribute to a transition between the HOMO and HOMO-2 ground state energy levels, as calculated for both Trp neutral and cationic radicals.^{28,29} Sinicropi and coworkers have calculated the absorption maximum for this HOMO-2 $\rightarrow$ HOMO transition (their $D_2 \rightarrow D_0$) as coincident with the shorter wavelength peak in the homogeneously broadened absorption spectrum of Trp radicals.^{28,29} Their calculations also yield an absorption transition at 320 nm for the cytochrome C peroxidase Trp 191 neutral radical (HOMO-3 $\rightarrow$ HOMO transition), but this absorbance was predicted to have a low oscillator strength. However, a weak shoulder at ~ 325 nm is observed in the absorption spectrum for rHSA (Fig. S3). Such absorption bands of low extinction coefficient have been observed in charge transfer complexes between indole, N^{α} -acetyl tryptophan and phthalimides.²⁰ Other DFT calculations for the visible absorption peaks found in Trp neutral radicals yield a HOMO-6 $\rightarrow$ HOMO transition at 456 nm,⁴⁸ which also allows for fluorescence.

The excitation spectrum for aqueous indole with 5 molar equivalents of NaOH, corresponding to the emission spectrum given in Fig. 5b, is shown in Figure 6 (blue line, short dash). The complete set of excitation spectra for the titration with NaOH are given in Figure S10. The excitation spectrum for Puro A in SDS micelles is also shown in Figure 6 (black line, long dash). Both excitation spectra are redshifted by 36 nm in Fig. 6 to align

them with the calculated homogeneously broadened vibrations that create the absorption spectrum of cytochrome C peroxidase Trp 191 neutral radical.²⁹ This calculated absorption spectrum was chosen for comparison over that for the neutral radicals, Cyt C peroxidase Trp 51•, Az48W• and ReAz108W•, because of the high resolution of the vibrational modes at the smallest homogeneous broadening factor, $\Gamma = 5$, but any of these other three could have been used in the ensuing comparison because the homogeneously broadened peaks are of similar intensity and location.^{28,29} The bottommost spectrum in Fig. 6 shows the absorption spectrum for Trp 191• created with the broadening factor, $\Gamma = 5$.²⁹ Individual vibrational modes are clearly visible. When $\Gamma = 100$ (Fig. 6, orange solid line), resolution is lost, and the broadened peaks are characterized by a maximum that coincides with the most intense vibration in the immediate spectral region. This calculated absorption spectrum is remarkably similar to the excitation spectrum of Puro A in SDS micelles: both the peak spacing and relative peak intensity coincide.

Because of this peak coincidence, the Puro A excitation spectrum (Fig. 6) was fit to Gaussian bandshapes so that peak wavelengths could be extracted and matched to calculated Trp radical vibrational modes. The Puro A excitation peaks that correspond to the vibrational 'fingerprint' region are given in Table 3, column 1. The vibrational modes of the Puro A excitation spectrum showed better correlation with the vibrational progression of the Trp neutral radicals than with the Trp radical cations of cytochrome C peroxidase.²⁹ For example, no correspondence could be found for the 1120.91 cm^{-1} and 1611.92 cm^{-1} peaks in either Trp radical cation progression. This deficit in vibrational mode correlation, coupled with the better excitation and absorption maxima wavelength fit with those for the absorption maxima of Trp neutral radicals are the reasons for choosing comparison with Trp neutral radical spectra over those for the Trp radical cation.

The peak located at 491.81 nm matches the low frequency modes with high Franck-Condon factors, ν_8 and ν_9 , of the Trp 191 neutral radical.²⁹ Assigning the excitation peak at 491.81 nm to these low frequency, high intensity modes, results in a 0-0 adiabatic origin of 493.36 nm for Puro A. Other calculated vibrational frequencies for Puro A can be found in Table 3, column 2. These vibrational modes are also matched to the calculated vibrational modes of high Franck-Condon factor for Cyt C peroxidase W191• and Az48W•,²⁸ and experimentally determined modes for Az48W•³⁸ and the *E. coli* photolyase Trp306•.⁵⁰ These corresponding modes are listed in Table 3, columns 3 to 6. For some Puro A peaks, more than one vibrational mode--those that are close in wavenumber---are listed due to the low resolution of the excitation spectrum.

Calculated vibrational modes for W191• that are close to the Puro A excitation peaks are ν_{23} (532.49 cm^{-1}), ν_{24} (548.31 cm^{-1}), ν_{49} (1120.99 cm^{-1}), ν_{60} (1364.27 cm^{-1}) ν_{70} (1590.40 cm^{-1}) and ν_{71} (1623.73 cm^{-1}), as given in Table 3, column 3. The visible resonance Raman spectrum for Az48W• has also been calculated,²⁸ and frequencies matched to the excitation spectrum peaks of the Puro A in SDS micelles as well. These are given in column 4 of Table 3. Matches are found for seven modes: W19•, W17•, W14•, W7•, W3•, W2•, and W1•.

The fluorescence excitation peaks for Puro A in SDS micelles can also be aligned with those obtained in visible resonance Raman experiments. The vibrational modes for the 514.5 nm excited resonance Raman spectrum of Az48W,³⁸ which correspond to the excitation spectrum peaks, are the same modes found for the calculated modes: W19-, W17-, W14-, W7-, W3-, W2-, and W1-. These vibrational mode frequencies are listed in column 5 of Table 3. Lastly, the fluorescence excitation peaks correlate with visible (527 nm excitation) resonance Raman peaks for the *E. coli* photolyase Trp306 neutral radical,⁵⁰ which are given in the last column of Table 3. Corresponding vibrational frequencies are found for modes W17-, W10-, W7-, W2- and W1-.

The alignment of fluorescence excitation peaks for the indole-NaOH complex (Fig. 6, blue short dashed line) with those of the calculated absorption spectrum for the Trp 191 neutral radical suggest that a similar vibrational analysis would yield an analogous correspondence with calculated and experimental vibrational modes for Trp neutral radicals. The greater intensity of the excitation peak at 467 nm relative to that for the Arg guanidinium reflects the stronger electrostatic interaction of Na⁺ with the indole π electrons. These results further reinforce the concept of a photophysical relationship between cation- π interactions and the Trp neutral radical.

CONCLUSIONS

The cation- π interaction for amine-Trp noncovalent complexes in a protein environment possesses charge-transfer properties, as predicted by theoretical studies. Electronic absorptive and emissive transitions in the visible region are the result of the orientation of the cation face-on relative to the indole plane. Reduced electron density in the indole HOMO provides for a HOMO-2 $\rightarrow$ HOMO visible absorption transition. The absorption band positions and intensities are sensitive to the specifics of molecular orientation and identity. The electronic spectra and vibrations extracted from fluorescence excitation spectra for cation- π interactions recapitulate those found for the Trp neutral radical even though the Trp has not been photoexcited to the radical state with UV light. The spectroscopic similarities discussed here imply some radical character for the cation- π interaction. A true radical exists when a single electron is entirely removed from the π system of the indole ring. The evidence presented demonstrates that electron density is removed from the indole ring, and is sufficient to impart the radical signature electronic absorption to the cation- π interaction. We predict that other cation- π molecular pairs will show their own subtle spectroscopic signatures in an easily identifiable and isolated part of the spectrum, as made evident by the fluorescence data for the Na⁺-indole complex and the Tyr-Trp dipeptide, thus enabling the spectroscopic characterization of cation- π interactions in the broad scope of biological systems in which they are found. Moreover, these results show that only one or two point mutations, constituting the appropriate cation-aromatic pair of native amino acids, can impart visible fluorescence to a protein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ma JC, Dougherty DA. *Chem Rev.* 1997; 97:1303. [PubMed: 11851453]
2. Dougherty DA. *Acc Chem Res.* 2013; 46(4):885. [PubMed: 23214924]
3. Dougherty DA. *Science.* 1996; 271(5246):163. [PubMed: 8539615]
4. Mahadevi AS, Sastry GN. *Chem Rev.* 2013; 113(3):2100. [PubMed: 23145968]
5. Daze KD, Hof F. *Acc Chem Res.* 2013; 46(4):937. [PubMed: 22724379]
6. Chan DI, Prenner EJ, Vogel HJ. *Biochim Biophys Acta.* 2006; 1758(9):1184. [PubMed: 16756942]
7. Jing W, Demcoe AR, Vogel HJ. *J Bacteriol.* 2003; 185(16):4938. [PubMed: 12897014]
8. Beene DL, Brandt GS, Zhong W, Zacharias NM, Lester HA, Dougherty DA. *Biochemistry.* 2002; 41:10262. [PubMed: 12162741]
9. Xiu X, Puskar NL, Shanata JAP, Lester HA, Dougherty DA. *Nature.* 2009; 458(7237):534. [PubMed: 19252481]
10. Van Arnem EB, Dougherty DA. *J Med Chem.* 2014; 57(15):6289. [PubMed: 24568098]
11. Mecozzi S, West AP, Dougherty DA. *J Am Chem Soc.* 1996; 118(9):2307.
12. Ansorg K, Tafipolsky M, Engels B. *J Phys Chem B.* 2013; 117(35):10093. [PubMed: 23924321]
13. Sherrill CD. *Acc Chem Res.* 2013; 46(4):1020. [PubMed: 23020662]
14. Khan HM, Grauffel C, Broer R, MacKerell AD, Havenith RWA, Reuter N. *J Chem Theory Comput.* 2016; 12(11):5585. [PubMed: 27682345]
15. Zhu WL, Tan XJ, Puah CM, Gu JD, Jiang HL, Chen KX, Felder CE, Silman I, Sussman JL. *J Phys Chem A.* 2000; 104:9573.
16. Aschi M, Mazza F, Di Nola A. *J Mol Struct THEOCHEM.* 2002; 587(1):177.
17. Kim KS, Lee JY, Lee SJ, Ha TK, Kim DH. *J Am Chem Soc.* 1994; 116(16):7399.
18. Lee JY, Lee SJ, Choi HS, Cho SJ, Kim KS, Ha TK. *Chem Phys Lett.* 1995; 232(1–2):67.
19. Caldwell J, Kollman P. *J Am Chem Soc.* 1995; 117:4177.
20. Carrion J, Deranleau D, Donzel B, KE, Moser P, Schwyzer R. *Helv Chim Acta.* 1968; 51:459. [PubMed: 5683976]
21. Matsunaga Y. *Bull Chem Soc Jpn.* 1973; 46:998.
22. Gartland G, Freeman G, Bugg C. *Acta Crystallogr Sect B.* 1974; 30:1841.
23. Goodnow TT, Reddington MV, Stoddart JF, Kaifer AE. *J Am Chem Soc.* 1991; 113(11):4335.
24. Moretto A, Formaggio F, Crisma M, Kaptein B, Broxterman Q, Toniolo C. *J Pept Res.* 2005; 65:15. [PubMed: 15686530]
25. Gallivan JP, Dougherty DA. *J Am Chem Soc.* 2000; 122:870.
26. Koebel, M., Sirimulla, S. Abstracts of Papers, 250th ACS National Meeting & Exposition. Boston, MA: 2015. p CIN-45
27. Padgett CL, Hanek AP, Lester HA, Dougherty DA, Lummis SCR. *J Neurosci.* 2007; 27(4):886. [PubMed: 17251430]
28. Bernini C, Andruniów T, Olivucci M, Pogni R, Basosi R, Sinicropi A. *J Am Chem Soc.* 2013; 135(12):4822. [PubMed: 23458492]
29. Bernini C, Arezzini E, Basosi R, Sinicropi A. *J Phys Chem B.* 2014; 118(32):9525. [PubMed: 25084495]

30. Roveri OA, Braslavsky SE. Photochem Photobiol Sci. 2012; 11(6):962. [PubMed: 22273601]
31. Görbitz CH, Hartviksen LM. Acta Crystallogr C. 2008; 64(Pt 3):o171. [PubMed: 18322347]
32. Zacharias N, Dougherty DA. Trends Pharm Sci. 2002; 23:281. [PubMed: 12084634]
33. Bachem, Inc. Analytical Data Sheet G-1530. L-Arg-L-Trp · 2HCl. Torrance, CA:
34. Woody, RW., Dunker, AK. Circular Dichroism and the Conformational Analysis of Biomolecules. Fasman, GD., editor. Plenum Press; New York: 1996. p. 109-157.
35. Solar S, Gtoff N, Surdhar PS, Armstrong DA, Singh A, Getoff N, Surdhar PS, Armstrong DA, Singh A. J Phys Chem. 1991; 95(9):3639.
36. Aubert C, Vos MH, Mathis P, Eker AP, Brettel K. Nature. 2000; 405:586. [PubMed: 10850720]
37. Miller JE, Gr dinaru C, Crane BR, Di Bilio AJ, Wehbi WA, Un S, Winkler JR, Gray HB. J Am Chem Soc. 2003; 125(47):14220. [PubMed: 14624538]
38. Shafaat HS, Leigh BS, Tauber MJ, Kim JE. J Am Chem Soc. 2010; 132(26):9030. [PubMed: 20536238]
39. Joly L, Antoine R, Allouche AR, Dugourd P. J Am Chem Soc. 2008; 130(42):13832. [PubMed: 18817390]
40. Shen X, Lind J, Merenyi G. J Phys Chem. 1987; 91:4403.
41. Wagenknecht HA, Stemp EDA, Barton JK. Biochemistry. 2000; 39(18):5483. [PubMed: 10820021]
42. Gill SC, von Hippel PH. Anal Biochem. 1989; 182:319. [PubMed: 2610349]
43. Truong TB. J Phys Chem. 1980; 84(9):960.
44. Basch H, Stevens WJ. J Mol Struct THEOCHEM. 1995; 338(1-3):303.
45. Mecozzi S, West AP, Dougherty DA. Proc Natl Acad Sci. 1996; 93(20):10566. [PubMed: 8855218]
46. Yagil G. Tetrahedron. 1967; 23:2855. [PubMed: 6047529]
47. Nowak M, Kearney P, Sampson J, Saks M, Labarca C, Silverman S, Zhong W, Thorson J, Abelson J, Davidson N, Schultz P, Dougherty D, Lester H. Science (80-). 1995; 268(5209):439.
48. Crespo A, Turjanski AG, Estrin DA. Chem Phys Lett. 2002; 365:15.
49. Stoll S, Shafaat HS, Krzystek J, Ozarowski A, Tauber MJ, Kim JE, Britt RD. J Am Chem Soc. 2011; 133(45):18098. [PubMed: 22007694]
50. Gurudas U, Schelvis JPM. J Am Chem Soc. 2004; 126(40):12788. [PubMed: 15469269]
51. Callis PR, Liu T. J Phys Chem A. 2004; 108:4248.
52. Eisenberg AS, Juszczak LJ. J Phys Chem B. 2014; 118(25):7059. [PubMed: 24882092]
53. Van Gilst M, Hudson BS. Biophys Chem. 1996; 63(1):17. [PubMed: 8981748]
54. Nanda V, Brand L. Proteins Struct Funct Genet. 2000; 40(1):112. [PubMed: 10813836]
55. Osysko AP, Muino PL. J Biophys Chem. 2011; 2(3):316.
56. Takeuchi H, Harada I. Spectrochim Acta, Part A. 1986; 42:1069.
57. Miura T, Takeuchi H, Harada I. Biochemistry. 1988; 27(1):88. [PubMed: 3349046]
58. Shafaat HS, Leigh BS, Tauber MJ, Kim JE. J Phys Chem B. 2009; 113(1):382. [PubMed: 19072535]
59. Okada A, Miura T, Takeuchi H. Biochemistry. 2001; 40(20):6053. [PubMed: 11352741]
60. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004; 25(13):1605. [PubMed: 15264254]

Figure 1.

Cation- π interactions in Trp-containing dipeptides. **a.** Intra- and interpeptide cation- π interactions between Tyr residues and the N-terminal amine in L-Tyr-L-Trp monohydrate. (682837.cif).³¹ **b.** Intra- and interpeptide cation- π interactions between Trp residues and the N-terminal amine in L-Trp-L-Ser monohydrate. (682836.cif) ³¹

Figure 2.

Visible absorption spectra for pink peptides, 1 mm path reflective optics. a. L-Arg-L-Trp [c]=280 mM, aqueous b. Puro A in 20 mM Tris, pH 7.4 [c]=22 mM c. L-Tyr-L-Trp [c]=56 mM, aqueous d. L-Trp-L-Ser, [c]=340 mM, aqueous. All peak positions are given in Table 1. Absorption bands have been offset on the ordinate for clarity. All spectra are on the same absorbance scale with the absorbance for the longer wavelength band ranging from 0.013 to 0.019. Inset: UV-vis spectrum of Puro A (200–600 nm).

Figure 3. Puro A bactericidal domain showing the W5-R6-W7 or π -cat- π motif, NMR in SDS micelles.⁷

Figure 4.
Lys 199 and Arg 218 amine distances to Trp 214 indole ring in rHSA (PDB 1AO6).

Figure 5.

Fluorescence emission spectra for titration of aqueous indole with equimolar aliquots of base, front face 467 nm excitation, 3 nm band pass. Bottommost spectrum is for indole neat. Emission increases with each additional aliquot. Spectra have been corrected for dilution. The signal noise centered at ~550 nm is due to subtraction of the water Raman peak. **a.** titration of NH_4OH , emission increases with pH: 10.57, 10.71, 10.81, 10.87, 10.91, 10.95, 10.98, 11.01, 11.03; [indole] = 7.5mM. **b.** titration of NaOH , emission increases with pH: 11.8, 12.1, 12.3, 12.4, 12.5; [indole] = 6.4mM.

Figure 6.

Experimentally determined fluorescence excitation spectra, front face detection for: indole $[c]=6.4$ mM, with 5 molar equivalents of NaOH (blue short dash line, maximum of (85K counts at 467 nm), 3 nm band pass, 505 nm emission wavelength; and Puro A in SDS micelles, $[c]=0.18$ mM (black long dash line, maximum of 142K counts at 492 nm). 2 nm band pass, 520 nm emission wavelength, average of 10 scans. Water Raman peak at 440 nm and baseline have been subtracted out for both excitation spectra. Calculated absorption spectrum for the neutral radical, Trp 191, from cytochrome C peroxidase: bottom trace, homogeneous broadening factor, $\Gamma=5$; $\Gamma=100$ (orange solid line).²⁹ Note the excitation spectra have been offset 36 nm to the red. Inset: absorption spectra for indole with 5x NaOH, 1mm path tray cell, max abs of 0.0011 at 502 nm, second peak at 462 nm.

Scheme 1.

Interaction of indole with Na and hydroxide ions. The Na cation is positioned above the benzyl ring of the indole ring.^{11,45} HOMO π -electron density is delocalized towards the Na ion, creating a partial electron deficiency on the indole ring. The hydroxide anion hydrogen bonds to the indole amine, creating a compensatory, stabilizing partial negative charge on the indole. At high $[\text{Na}^+]$, a second Na^+ may be positioned on the opposite face of the benzyl ring (not shown). The resulting HOMO electron deficiency allows for the observed visible absorption from the lower energy HOMO-2 level.²⁹ Fluorescence is possible because the absorption transition is vertical. Scheme created using the Chimera software program.⁶⁰

Table 1
Absorption and Fluorescence Data for Cation- π Complexes in Indole, Trp-Containing Dipeptides, Puro A and rHSA

Peptide (state)	absorption peaks (nm)	Extinction coefficient (M-cm) ⁻¹	Emission maximum (nm)	lifetimes (ns)	Amplitude (%)	Weighted average lifetime (ns)	R ²	Quantum yield
Indole-NaOH pH 12	462 502		498					
Indole-NH ₄ OH pH 11	-		512					
L-Arg-L-Trp• 2HCl (aq) ¹ (ss) ²	470 494 -		503 515	9.17 2.459 0.676	3.72 44.09 52.20	1.78	1.04	
L-Arg-L-Trp (aq) (ss)	472 504 -		519 519	8.06 2.814 0.888	5.42 60.48 34.10	2.44	1.04	
L-Tyr-L-Trp (aq) (ss)	470 503 -		522 529					
L-His-L-Trp (aq) (ss)	472 492 -		501 510					
L-Trp-L-Ser (aq) (ss)	469 502 -		506 505					
Puro A (buffer)	467	1696	515					

Peptide (state)	absorption peaks (nm)	Extinction coefficient (M-cm) ⁻¹	Emission maximum (nm)	lifetimes (ns)	Amplitude (%)	Weighted average lifetime (ns)	R ²	Quantum yield
(SDS)	502	2060	520	4.228	69.55	3.60	1.08	
	470			2.180	30.45			
	501							
(ss)	-		506					
rHSA (buffer)	467	655	515	12.1	20.99	5.17	1.16	0.0070
	504	867		4.38	53.78			
				1.08	25.23			
				7.81 ³	23.21 ³			
				4.38	58.27			
				1.49	18.52	4.64	1.19	0.012 ³

¹ _{aq} = aqueous

² _{ss} = solid state

³ _{UV} excitation

Table 2

Absorption Maxima for Experimental and Theoretical Transient Tryptophan Neutral Radicals

Trp Containing Molecule	Peak Maxima (nm)	
Trp, acidic solution ¹	532	
Trp, pH 8–10 ²	510	
Lys-Trp-Lys, DNA-bound ³	500	
ReAz108W·ZnII ⁴	512	536
ReAz48W. ⁵	486	516
ReAz108W. ⁵	510	537
Az48W. ⁶	464	494
Cyt C peroxidase Trp 51 ⁷	475	511
Cyt C peroxidase Trp 191 ⁷	493	528

¹
ref. 26²
ref. 29³
ref 41⁴
ref 37⁵
ref 38⁶
ref 28⁷
ref 29

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Normal modes for the cation- π interaction in Puro A in SDS micelles from the vibrationally-resolved fluorescence excitation spectrum with calculated and experimental neutral Trp radical normal modes.

Table 3

Peak (nm)	Vibrational mode frequency (cm ⁻¹)				
491.56	74.22 ¹	37.69 (ν_8) ² 64.39 (ν_9)	-	-	-
481.04	518.37	532.49 (ν_{23}) 548.31 (ν_{24})	547 (W19) ³	542 (W19) ⁴	-
472.33	901.72	-	841 (W17 $\cdot$)	838 (W17 $\cdot$)	826 (W17 $\cdot$) ⁵
467.49	1120.91	1120.99 (ν_{49})	1125(W14 $\cdot$)	1111(W14)	1080 (W10 $\cdot$)
462.56	1349.64	1364.27 (ν_{60})	1353 (W7 $\cdot$) 1367(W3 $\cdot$)	1332 (W7 $\cdot$) 1342(W3 $\cdot$)	1342/1333 (W7 $\cdot$)
457.00	1611.92	1590.40 (ν_{70}) 1623.73 (ν_{71})	1585 (W2 $\cdot$) 1619 (W1 $\cdot$)	1559 (W2 $\cdot$) 1588 (W1 $\cdot$)	1557 (W2 $\cdot$) 1590 (W1 $\cdot$)

¹ 493.36 nm = 0-0 wavelength.

² Bernini et al.,²⁹ calculated normal modes with largest dimensionless normal coordinate displacement for D0 $\rightarrow$ D2 transition of Trp 191 $\cdot$.

³ Bernini et al.,²⁸ calculated vibrational modes for Az48W $\cdot$.

⁴ Shafaat et al.,³⁸ visible resonance Raman modes for Az48W $\cdot$, 514.5 nm excitation.

⁵ U. Gurudas and PM Schelvis,⁵⁰ visible resonance Raman modes for Trp306 neutral radical for *E. coli* photolyase.

Table 4

UVRR (229 nm) W17 peaks for indole, L-Trp and cation- π containing peptides and protein.

	W17 (cm ⁻¹)
L-Trp ¹	879
L-Arg-L-Trp·2HCl ²	879
Puro A ³	878
rHSA ⁴	876
Indole-NaOH, 1:5	879

¹ aqueous

² 80% acetic acid

³ SDS micelles

⁴ 25mM Tris, 25 mM MES, pH7.0