Methods

Synthesis and purification of PRTNs.

Peptides were prepared and modified on resin adapting 9-fluorenylmethoxycarbonyl (Fmoc) solid phase peptide synthesis methods previously described[1]. di-C_{16}-NLS was synthesized by conjugating a synthetic lipid to the N-terminal amine[2]. Fluorescently labeled di-C_{16}-NLS was synthesized by conjugating 5(6)-carboxytetramethyl rhodamine to the ε-amine of a 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) protected lysine. The bZip peptide was purchased protected on the resin (Anaspec Inc.). The peptide amphiphile was prepared by conjugating the synthetic lipid to the ε-amine on a Dde protected Lys at the C-terminus of the peptide. Fluorescently labeled di-C_{16}-bZip, was prepared by conjugating 5,6-carboxy-fluorescein to the N-terminal amine. All peptide amphiphiles were purified on a C_8 preparative reverse-phase high performance liquid chromatography (Shimadzu) to above 95% purity. Materials were analyzed on a C_8 analytical column, and identity determined using either electrospray ionization-time of flight (ESI-TOF) or matrix-assisted laser desorption ionization (MALDI) mass spectrometry.

The dendrimer was synthesized according to a previously published procedure[3]. Briefly, a well-defined 5th generation dendrimer bearing azide end-groups was rapidly prepared starting from a trifunctional alkene core by sequential thiol-ene and copper catalyzed azide-alkyne cycloaddition (CuAAC) reactions. The alkyl functionalized dendrimer with a hydrophobic exterior was synthesized via a CuAAC click reaction between the 5th generation azide-functional dendrimer and 1-decyne.

Peptide amphiphiles were adsorbed onto the dendrimer via a solvent exchange method. For the 50 nm PRTNs, 1.4 nmol of dendrimer and 27.4 nmol of PA were first dissolved in 75 μl of tetrahydrofuran (THF) and 25 μl of methanol. After vortexing to mix, 1 ml of room temperature ultrapure water was added. The samples were then vortexed again and the solvent evaporated

under nitrogen gas until the final volume of reached less than 1 ml. Ultrapure water was then added until the total volume reached 1 ml, giving a final dendrimer concentration of 1.4 μM and PA concentration of 27.4 μM. Solutions were filtered through a 0.2 μm syringe filter before use. This concentration ratio of approximately 20 PAs per dendrimer particle was found to form the smallest PRTNs. The relationship between PRTN size and absolute dendrimer concentration was also tested. PRTN solutions prepared at a concentration of 1.4 μM total dendrimer resulted in smaller particles than solution concentrations of 5 μM and 10 μM dendrimer, even when the PA:dendrimer ratio was held constant at 20:1. PRTN concentration and salt concentration adjustments post-hydration did not affect PRTN size. Micelles were prepared using the same solvent exchange method described for the PRTNs for consistency. PRTNs and micelles were equilibrated for 24 hours after preparation before experiments were performed.

Characterization of PRTNs.

Hydrodynamic radii of PRTNs were measured by dynamic light scattering on a Viscotek 802 (Malvern Instruments Ltd) at a scattering angle of 90°.

All samples used for cryogenic transmission electron microscopy (cryo-TEM) imaging were prepared in a controlled environment at 24°C and 100% humidity (FEI Vitrobot Mark IV). A 3.5 μL droplet of the sample, at a concentration of 1-10 mg/mL, was pipetted onto a glow discharged lacey carbon coated copper grid. The sample was blotted once with filter paper for 2-3 seconds before being plunged into liquid nitrogen cooled liquid ethane. The samples were placed in a cryo-holder and were kept below -170°C throughout imaging. Samples were imaged at 200kV using low dose mode. Images were recorded digitally and were analyzed using the Gatan Digital Micrograph software. Concentrated PRTNs were prepared as described above and concentrated to 1-10 mg/ml by evaporating the water under a stream of nitrogen gas.

Critical aggregation concentrations (CACs) were determined for di-C_{16}-NLS in the presence and absence of dendrimer using the pyrene fluorescence method[4]. The pyrene

working solution was prepared at 2 μM and PA concentrations ranged from 250-0.01 μM. Measurements were taken on a plate reader (Tecan Infinite M200) one hour after the series of dilutions were prepared in 96-well plates. The pyrene was excited at 335 nm and fluorescence emission was measured at 373 nm and 393 nm. The ratio of fluorescence intensities at 393 nm and 373 nm (I_3/I_1) was plotted. The critical aggregation concentration is indicated by a change in slope in this data. Quantitatively, this was determined by fitting the two data subsets with a trendline and calculating the concentration at their intersection.

Circular dichroism (Jasco J-815) was used to determine the secondary structure of the bZip peptide and PAs in solution above and below the critical micelle concentration and on the surface of the dendrimer. Samples were prepared as previously described at a peptide concentration of 27.4 μM and a dendrimer concentration of 1.4 μM in water. Solution volumes of 300 μl were used in a 1 mm path quartz length cuvette and scanned from 260 nm to 190 nm. A water baseline was subtracted from the data and then converted to mean residue ellipticity. An average of 5 scans at 20°C is reported.

FRET was employed to analyze the co-assembly of the bZip and NLS PAs. The mixed PRTNs were prepared via solvent exchange at a final solution concentration of 13.7 μM rhodamine-labeled di-C_{16}-NLS PA, 13.7 μM fluorescein-labeled di-C_{16}-bZip PA, and 1.4 μM dendrimer. As controls, 13.7 μM rhodamine-labeled PRTNs and 13.7 μM fluorescein-labeled PRTNs were prepared separately (each with 1.4 μM dendrimer) and measured separately. FRET experiments were performed with 475 nm as the excitation wavelength and emission intensities scanned from 500—650 nm (Jasco FP-6500 Spectrofluorometer). Experiments were performed in duplicates.

DNA binding.

Complementary oligonucleotides of 30 base pairs were purchased (Integrated DNA Technologies) with one of the strands having a 3’ modification of a tetramethylrhodamine label.

The 5' → 3' sequence was GAAGCAGAACCATGACTCATGCGCTGAAGC. Strands were annealed at 95°C in tris-EDTA buffer (pH 8.0) for 5 minutes in equimolar amounts with their complements and allowed to cool slowly resulting in a final concentration of 50 μM dsDNA.

DNA for the binding experiments was prepared at a concentration of 66.7 nM strands in 10 mM concentration of phosphate buffer (1x) at pH 7.4. After the addition of PAs or PRTNs to the DNA, solutions were vortexed and allowed 2 hours to equilibrate. Rhodamine anisotropy was then measured at an excitation wavelength of 552 nm and emission at 584 nm at 25°C (Jasco FP-6500 Spectrofluorometer). The anisotropy value was calculated in the software with the equation

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

Data reported are an average of five anisotropy measurements taken at each bZip concentration from two separate stock solution preparations.

Atomic force microscopy was performed on a Veeco Dimension 3000 equipped with a STMP tip to visualize the DNA bound bZip PRTNs. 25 μl of 1 μM calf thymus DNA (ctDNA) and 5 μl of 27.4 μM PRTN stock were mixed in 10 mM Mg^{2+}, cast on freshly cleaved mica and incubated for 10 minutes, rinsed gently with ultrapure water, and dried gently under a N_2 stream. The samples were imaged within 20 minutes.

Cell uptake and imaging.

PRTNs and micelles were evaluated for their uptake in Hela cells. Cells were plated at a density of 10,000/cm², and experiments performed between 12-16 hours later. PRTNs were prepared at with a 9:1 ratio of fluorescein-labeled bZip PA:NLS PA. The final solution consisting of 18.2 μM PA total (either in micelles or on PRTNs) were introduced to the HeLa cells. After two hours the cells were washed four times with PBS, then fixed with 4% paraformaldehyde in PBS and rinsed

four times again in PBS. 100 μl of PBS was added to each well for imaging, which was performed on a Nikon TE200 microscope.

Kinetic stability studies.

The kinetics experiment monitored the fluorescence intensity of fluorescein over time, where the fluorescence of aggregated PAs (in micelles and on dendrimers) was quenched. As monomers desorbed from the micelles or dendrimer to interact with sites on the BSA, the fluorescence intensity of the solution increased because the fluorophores on the desorbed monomers were no longer quenched[5]. Temperature was controlled and kept constant throughout the experiment with a circulating water bath, and all micelle, PRTN, and BSA solutions were incubated at the proper temperature for 30 minutes prior to mixing. To prepare the samples, 108 μl of 27.4 μM fluorescein-labeled bZip PA micelles or PRTNs in water were mixed with 12 μl of 10x phosphate buffered saline (PBS, pH 7.4) for a resulting concentration of 24.7 μM PA in 1x PBS. Next, 30 μl of 4 x 10^{-3} M BSA in PBS was added to the cuvette and mixed with a pipette (final concentration of PA = 19.7 μM, BSA = 800 μM; about 40x molar excess of BSA) and fluorescence was measured immediately (excitation = 490 nm, emission = 530 nm; Jasco FP-6500) with this intensity used as time = 0 and fraction dissociated = 0. Subsequently, fluorescence was measured once every 30 seconds. At the end of the experiment a small volume of Triton-X (molar ratio of Triton-X:PA = 115:1) was added to dissociate the micelles or PRTNs and determine the value at which 100% of the fluorescein on the peptide amphiphiles was unquenched. Fraction dissociated (F) was then calculated at each time point by:

$$F = \frac{I_{(time=t)} - I_{(time=0)}}{I_{(Triton-X\ addition)} - I_{(time=0)}}$$

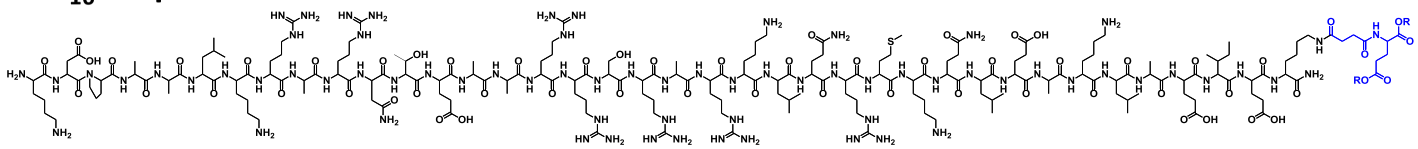
where I represents fluorescence intensity.

Three control experiments were performed:

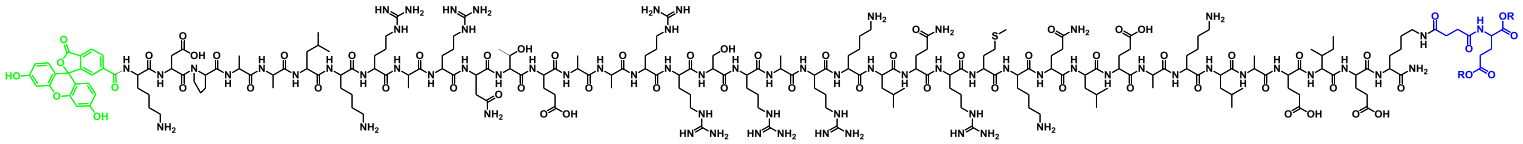
- The fluorescence of the micelle and PRTN samples was monitored over time with no BSA addition, and showed that fluorescein did not photobleach under the experimental conditions.
- 30 μl of PBS was added instead of 30 μl BSA and there was no change in fluorescence intensity, proving that micelle breakup was due to the presence of BSA and not dilution of the micelles or PRTNs.
- Only 10% of the peptide amphiphile monomers in the micelle or PRTN were tagged with fluorescein and therefore unquenched, which showed no increase in fluorescence over time after BSA addition. This verified that BSA did not change the fluorescence of fluorescein over time and the fluorescence increase in the other experiments was due to the dequenching caused by monomer desorption from the micelles or PRTNs.

(a) Peptide Amphiphiles

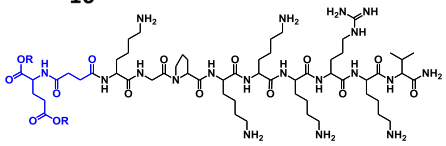
di-C_{16}-bZip



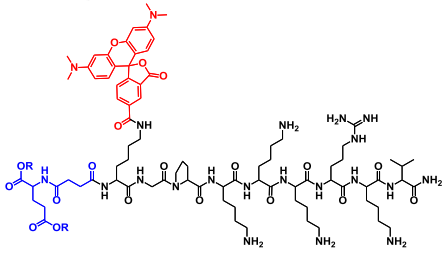
di-C_{16}-bZip-fluorescein

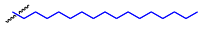


di-C_{16}-NLS



di-C_{16}-NLS-rhodamine



R: 

(b) Dendrimer

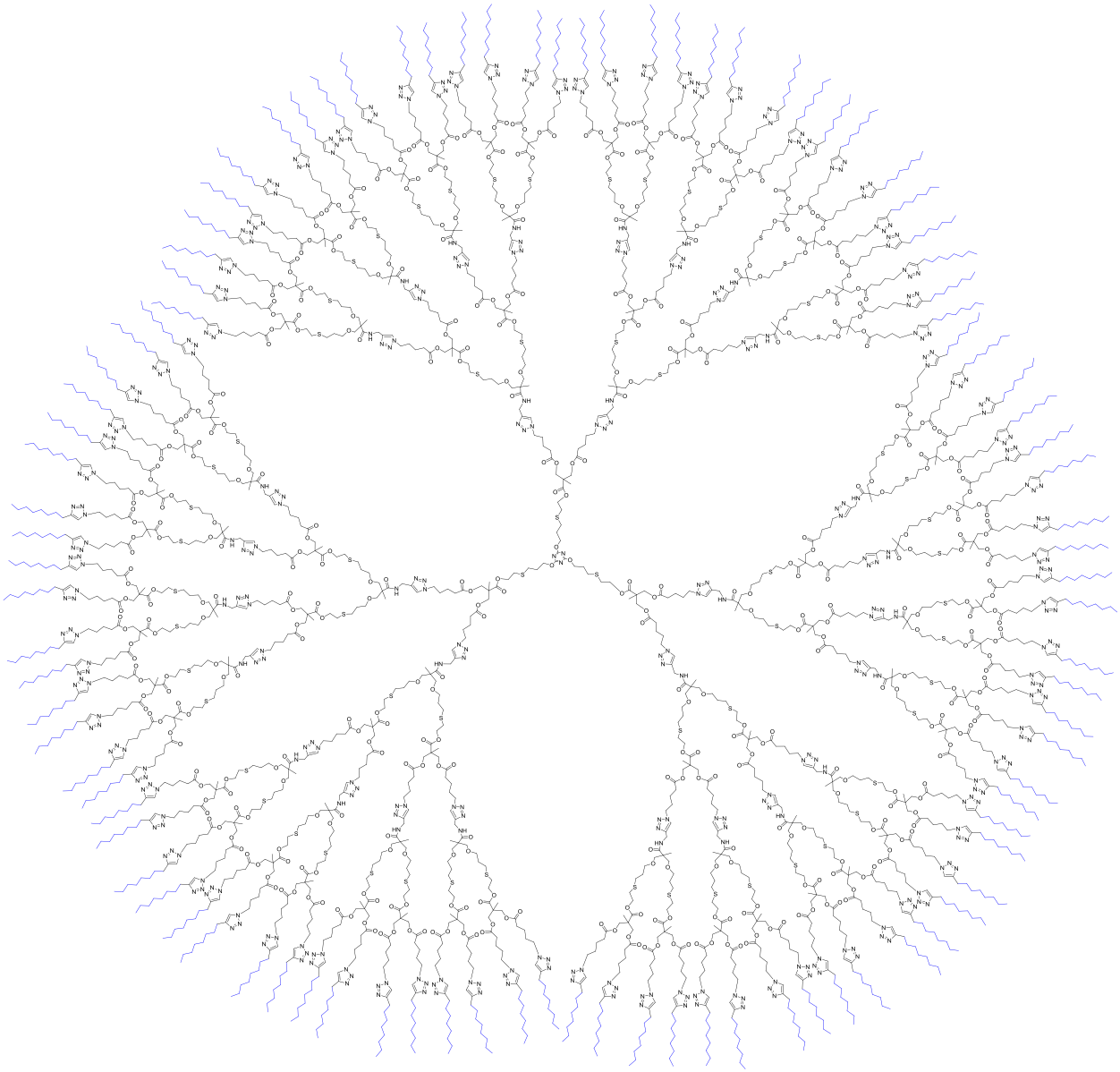


Figure S1. Chemical structures of the building blocks used in self-assembly. (a) Structure of peptide amphiphiles under investigation. Peptide sequence of bZip is KDPAALKRARNTEAARRSRARKLQRMKQLEAKLAEIEK and NLS sequence is KGPKKKRKV. (b) 5th generation dendrimer template rapidly synthesized using an orthogonal growth approach employing thiol-ene and azide-alkyne cycloaddition reactions. The hydrophobic moieties that interact during self-assembly are shown in blue.

	mass expected	mass found	method
di-C_{16}-bZip	5107.1	5108.1	MALDI
di-C_{16}-bZip-fluorescein	5465.2	5472.5	MALDI
di-C_{16}-NLS	1744.3	1746.3	MALDI
di-C_{16}-NLS-rhodamine	2156.8	2156.3	ESI-TOF

Table S1. Mass spectrometry results for the PA building blocks used in this work.

(a) (b)

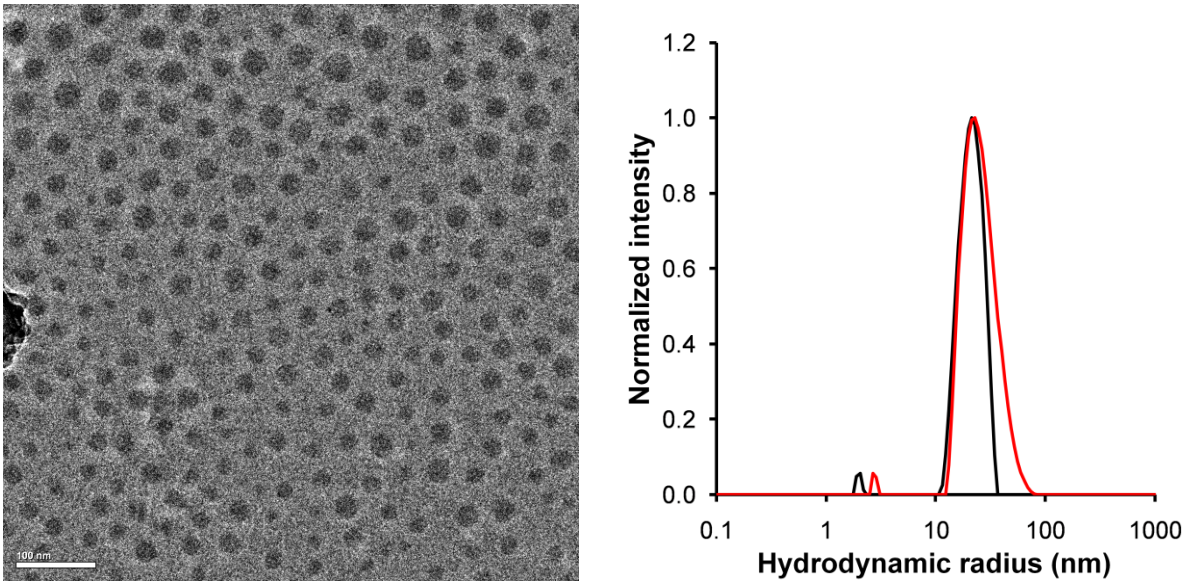


Figure S2. (a) Additional cryo-TEM image of NLS PRTNs (scale bar = 100 nm)**.** (b) DLS data showing the hydrodynamic radii of bZip PRTNs (—) and NLS PRTNs (—).

(a)

	r_H (nm) from DLS	
PA:dendrimer ratio	*NLS*	*bZip*
288:1	86.3 ± 25.3	N/A
96:1	50.9 ± 8.1	N/A
48:1	N/A	45.1 ± 17.1
20:1	21.5 ± 5.1	26.6 ± 9.6
10:1	24.5 ± 3.7	26.1 ± 4.9

Estimation of the number of peptides on the surface of PRTNs. For PRTNs with an average diameter of ~50 nm, the stoichiometric ratio of PA:dendrimer was 20:1. Considering that the diameter of a single dendrimer is 10 nm and the length of a peptide is 5 nm, the diameter of the hydrophobic core is ~40 nm. An approximation of the functionalization of each PRTN can be found based on two assumptions:

1.) Dendrimers pack into the PRTN cores as hard spheres with a void fraction of 0.5.

2.) 10% of PAs remain in solution (due to the CAC, aggregated PAs are in equilibrium with monomeric PAs in solution) and 5% of PAs are lost to filtration or trapped in PRTN cores.

Accounting for these assumptions, there are approximately 500 PAs on the surface of an average sized PRTN. This estimate is confirmed by comparing the PRTNs to a known system. The aggregation number of a small spherical PA micelle with roughly a 12 nm diameter is 80 to 100. Knowing that surface area is proportional to the square of the radius and assuming that the PA density is comparable on the surface of small spherical micelles and PRTNs, this approach gives a functionalization of 10^2-10^3 PAs on the surface of each PRTN (which agrees with the stoichiometric calculation).

(b)

	r_H (nm) from DLS		
PRTN	*Week 1*	*Week 2*	*Week 3*
bZip	26.6 ± 9.6	28.5 ± 6.7	29.3 ± 9.2
NLS	21.5 ± 5.1	26.1 ± 11.2	22.4 ± 1.7

Table S2. (a) PRTN sizes were determined by PA:dendrimer ratios. (b) PRTNs (PA:dendrimer ratio of 20:1) were stable for at least three weeks.

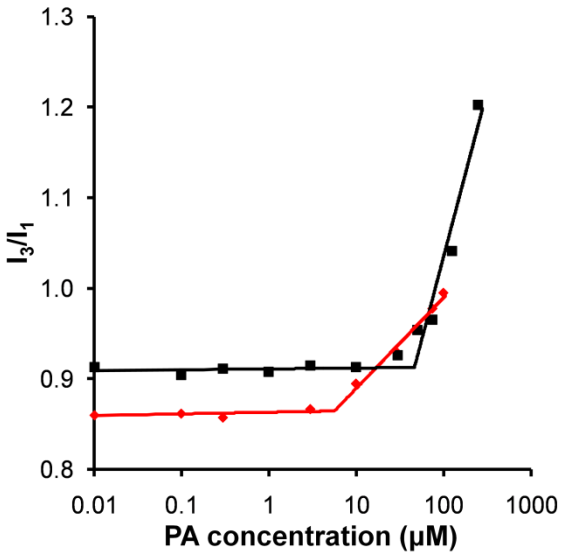
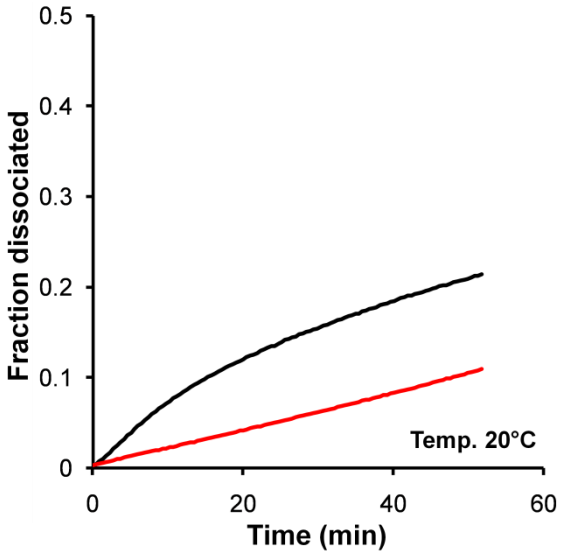


Figure S3. The critical aggregation concentration of NLS PRTNs (—) is 5.6 μM and of NLS vesicles (—) is 60.4 μM.

(a)



(b)

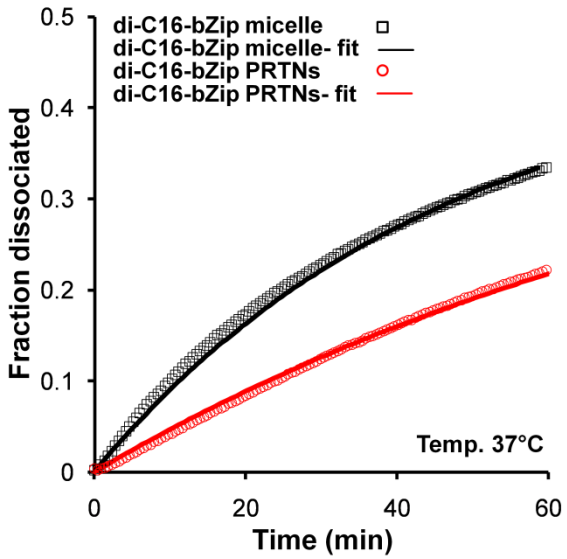


Figure S4. Dissociation kinetics at room and physiological temperature both demonstrate the enhanced stability of bZip PRTNs (—) over micelles (—). (a) Dissociation kinetics at 20°C. (b) Results from fitting 37°C data in the text Figure 2 with the equation: $I(t) = [I(0) - I(\infty)]e^{-kt} + I(\infty)$. *k* in this equation is the desorption rate constant. Fitting the data gives constants of $k^{-1} = 46.3\ min$ for bZip micelles and $k^{-1} = 92.6\ min$ for bZip PRTNs.

The kinetics experiment and data analysis are based on the following assumptions:

- PA desorption from the micelle or PRTN to BSA is the rate limiting step- much slower than PA exchange between other micelles or PAs in solution.
- Fluorescence increase is only attributed to PA capture by BSA.
- All PAs are able to leave the micelle/PRTN.
- There is no change in fluorescence intensity when a PA in the micelle/PRTN exchanges with one free in solution.
- PAs adsorbed onto BSA are not quenched.

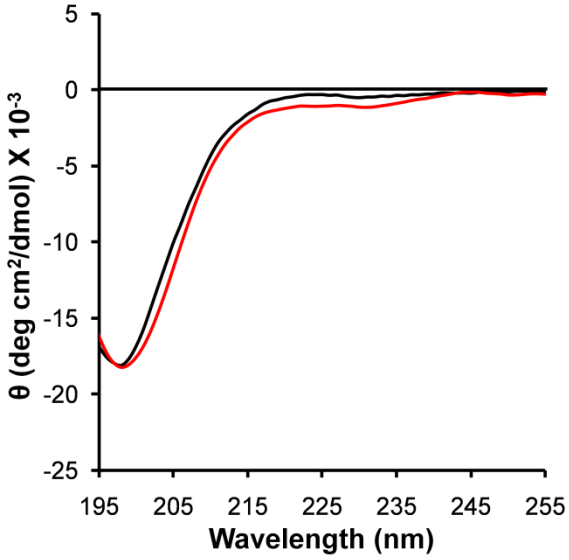


Figure S5. Circular dichroism spectra of NLS PRTNs (—) and NLS vesicles (—). Both exhibit random coil conformation, indicating that no change in secondary structure resulted between the PAs in the vesicles and PRTNs.

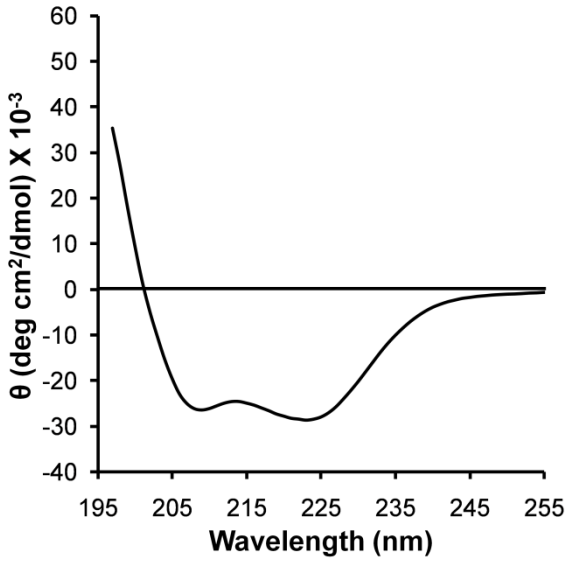


Figure S6. CD curve for the bZip PRTN in the presence of 30 bp DNA. The peptides displayed on the bZip PRTN maintain their helical structure as they bind DNA.

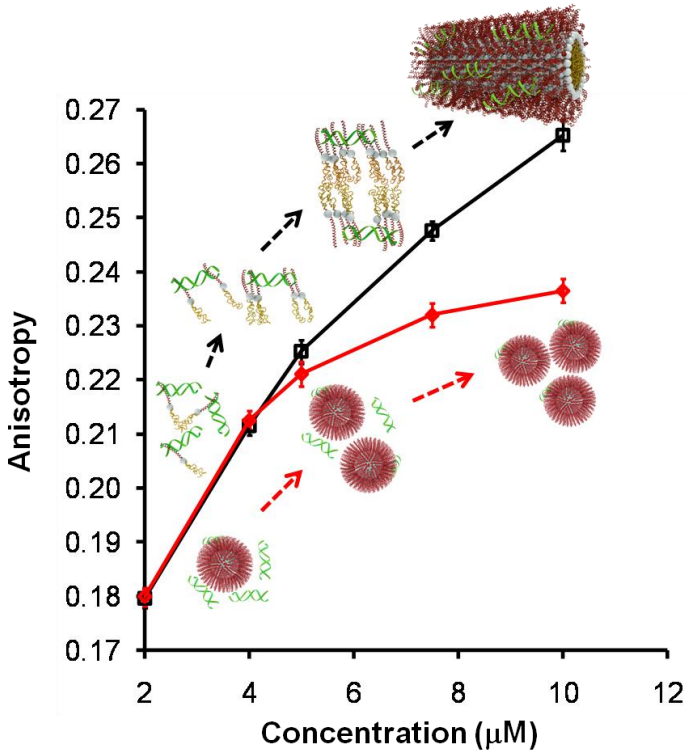


Figure S7. Anisotropy curve for the bZip PRTN binding to 30 bp DNA, with an illustrative description of the data.

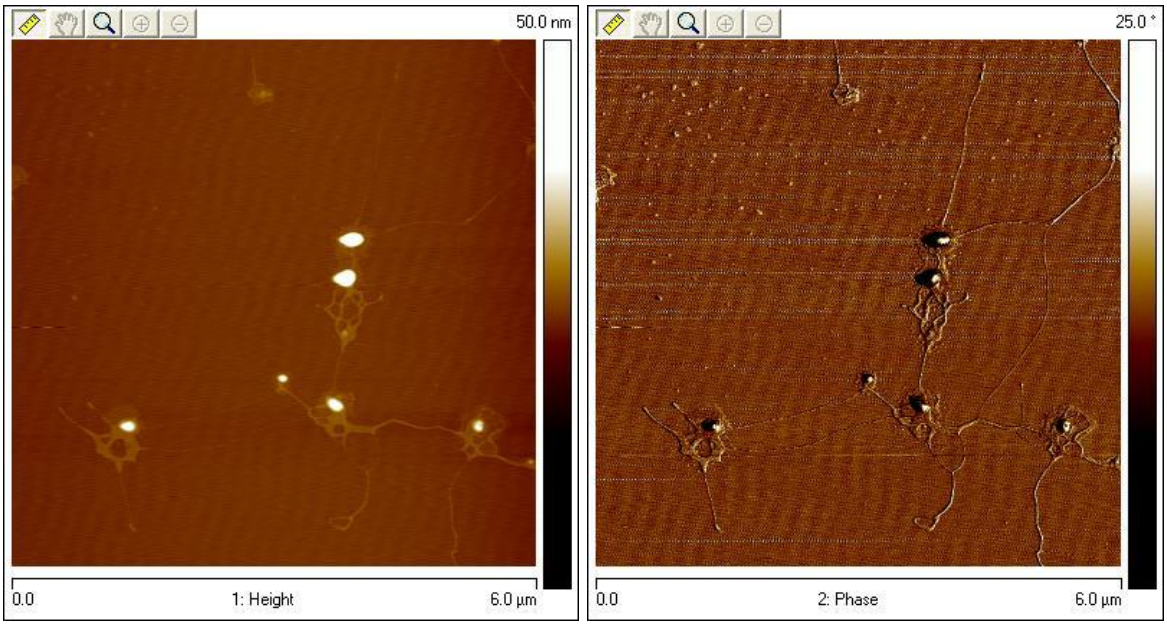


Figure S8. Additional AFM images of bZip PRTNs bound to ctDNA. (a) Height image. (b) Phase image. The PRTNs are spherical and bound to the DNA, not the surface.

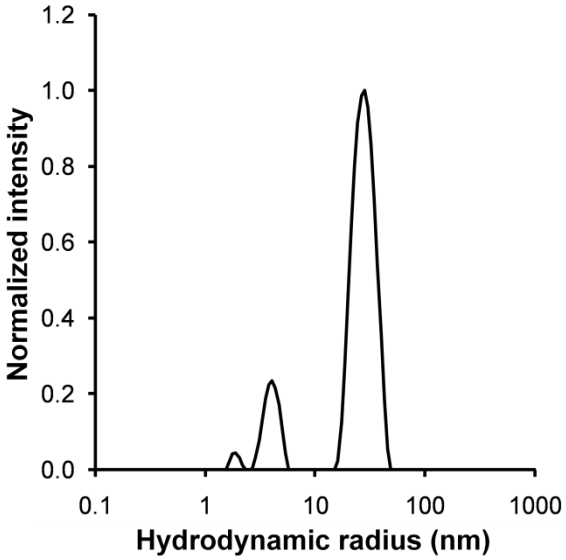
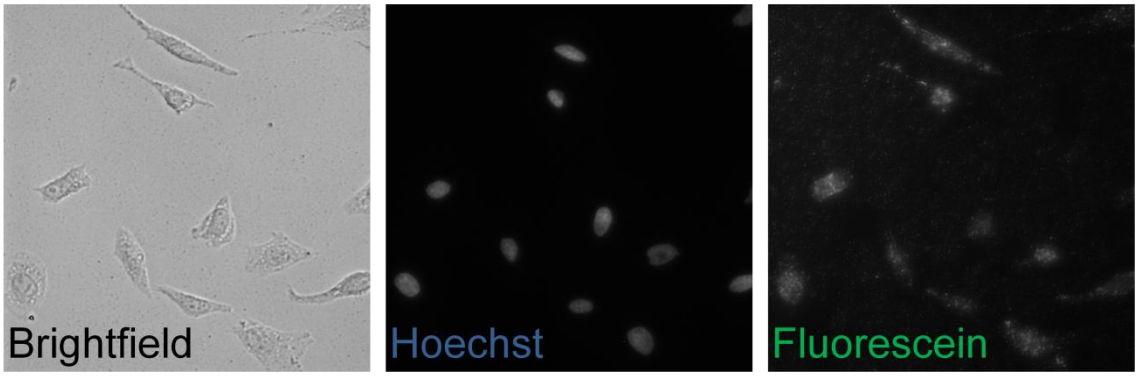


Figure S9. The DLS data showing the hydrodynamic radius of the mixed PRTNs (50% bZip PA and 50% NLS PA) to be 28.3 nm ± 6.2 nm.

(a)



(b)

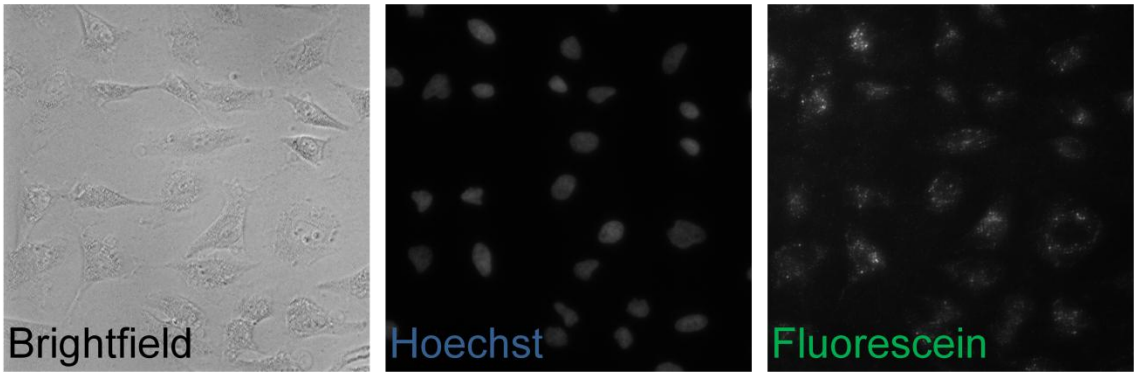


Figure S10. Three separate channels for cell images. (a) Mixed micelles. (b) Mixed PRTNs. The micelles and PRTNs have a 9:1 ratio of bZip-FAM PA:NLS PA.

References

(1) Merrifield, R. B. *J Am Chem Soc* **2002**, *85*, 2149.

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