Neurofibrillar tangle surrogates: Histone H1 binding to patterned phosphotyrosine peptide nanotubes

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ABSTRACT: Living cells contain a range of densely phosphorylated surfaces, including phospholipid membranes, ribonucleoproteins, and nucleic acid polymers. Hyper-phosphorylated surfaces also accumulate in neurodegenerative diseases as neurofibrillar tangles. We have now synthesized and structurally characterized a precisely patterned phosphotyrosine surface and establish this assembly as a surrogate of the neuronal tangles by demonstrating its high affinity binding to histone H1. This association with nucleic acid binding proteins underscores the role such hyper-phosphorylated surfaces may play in disease and opens functional exploration into protein/phosphorylated surface interactions in a wide range of other complex assemblies.

Tauopathies, including Alzheimer’s (AD) and Pick’s diseases, are characterized by the cytoplasmic accumulation of densely phosphorylated neurofibrillar tangles of the microtubule-associated tau protein.¹³ The recent identification of specific ribonucleoprotein co-localization with hyper-phosphorylated tau aggregates implies that co-aggregation or even cross-seeding events may be etiologically significant in AD by impacting RNA processing.⁴ Here we use the paracrystalline phase of the Aβ peptide to develop a neurofibrillar tangle surrogate with a precisely patterned phosphopeptide surface and use it to evaluate histone H1 binding.

Aβ(16-22), Ac-KLVFFAE-NH₂⁵ and its congener Ac-KLVFFAL-NH₂, (E22L), assemble as peptide bilayer nanotubes⁶⁻⁷ (Fig 1A) with diameters of 54 ± 3⁶ and 32 ± 5 nm, respectively (Fig. S1). The peptides in each leaflet of the peptide bilayer are arrayed as β-sheets having anti-parallel out-of-register strand arrangements, placing the N-terminal residues outside the H-bonded array.⁶ The positively-charged lysine side chains are in rows running the length of the tube and repeat every nanometer on each leaflet face.⁶

Reasoning that these robust, β-sheet rich, cross-β peptide assemblies might also accommodate phosphorylated residues along the leaflet surface, the phosphotyrosine Ac-(pY)LVFFAL-NH₂ peptide (K6pY)(E22L) was synthesized and conditions were found (40% MeCN/H₂O, 15 mM triethylammonium acetate at neutral pH) where nanotubes assemble with diameters of 32 ± 3 nm (Fig. 1E, Fig. S1).

Figure 1. (A) Model for (K6pY)(E22L) bilayer nanotubes with each leaflet composed of anti-parallel out-of-register β-sheets. The N-terminal phosphotyrosine (pY) repeats every nanometer along each row. (B-G) TEM micrographs of salt-induced bundling of (E22L) (top) and (K6pY)(E22L) (bottom) nanotubes. Left panels (B, E) without additional salts, middle panels (C, F) 2 hr after Na₂SO₄ addition, and right panels (D, G) 2 hr after MgCl₂ addition. Peptide/salt molar ratio 1:4.5 and scale bars are 200 nm.
The (K6pY)(E22L) nanotubes exhibit a circular dichroism (CD) ellipticity minimum at 225 nm (Fig. S2), most consistent with β-sheets. Powder X-ray diffraction (XRD) reflections with d-spacings of 4.7 Å and 10.3 Å (Fig. S3) can be assigned as β-strand and β-sheet reflections respectively within a cross-β assembly. FT-IR spectra (Fig. S4) contain a strong amide-I absorption band at 1623 cm⁻¹, further supporting H-bonded β-sheet structures, and a weak band at 1693 cm⁻¹ consistent with antiparallel strand arrangements. The out-of-register antiparallel organization is confirmed via solid-state NMR 13C{15N}REDOR measurements on [1-13C]Val8,[15N]Ala21-(K6pY)(E22L) assemblies, assigning Val8 as H-bonded to Ala21 on adjacent strands with 13C-15N distances of 4.2±0.2 and 5.8±0.2 Å and a 13C-15N angle of 156° (Fig. S5), identical to Aβ(16-22) and (E22L) tubes. As shown in Fig 1A, AFM (Fig S6) measurements are consistent with E22L and (K6pY)(E22L) tubes each maintaining the ~4 nm thick wall of a cross-β peptide bilayer. The anti-parallel β-sheet registry then places the phosphorylated residues on inner and outer tube surfaces and within the bilayer interface, establishing that this peptide bilayer is indeed capable of accommodating polar phosphates at the leaflet interface (Fig 1A).

Salt-induced aggregation was used to evaluate nanotube surface charge. Induction follows the Hofmeister series, with SO₄²⁻ binding (E22L) nanotubes but not (K6pY)(E22L) nanotubes (Fig. 1C,F), consistent with (E22L) having positively charged surfaces and (K6pY)(E22L) having negatively charged surfaces. Similarly Mg²⁺ only bundes (K6pY)(E22L) nanotubes (Fig. 1D, G). Further, citrate coated negatively-charged gold nanoparticles organize specifically along the surfaces of the (E22L) assemblies, while positively-charged gold nanoparticles (functionalized with (1-mercaptoundecyl)-N,N,N-trimethylammonium bromide) coat only the (K6pY)(E22L) assemblies (Fig S7). Together, these results are consistent with (K6pY)(E22L) having negatively charged surfaces and (E22L) nanotubes having positively charged surfaces.

Enhanced-electrostatic force microscopy (EFM) supports these assignments, and allows mapping of the charge distribution, as with other peptide assemblies and doped regions in semiconductors. Figure 2A shows the partially dried (E22L) assemblies as repulsive (white) to a positive bias tip while the (K6pY)(E22L) assemblies (Fig 2B) are attractive (dark) along the entire length of each assembled nanotube. These electrochemical potential measurements precisely map the assembled phases and the analysis appears independent of counter-ion as the same EFM images are obtained with gold nanoparticles (data not shown) and protein (Fig. S8). Together these spectroscopic and scanning probe analyses define a unique hyperphosphorylated peptide nanotube.

Histone H1 accumulates in the cytoplasm of neurons and astrocytes in areas impacted by neurodegenerative disease and serves as a prototypical nucleic acid binding protein for initial evaluation of our new phosphorylated nanotube surfaces. The addition of histone H1 to (K6pY)(E22L) nanotubes does not disrupt the assemblies as visualized by TEM (Fig 3A) and two-photon fluorescence images in Figure 3 show Alexa 488 fluorophore labeled calf thymus histone H1 alone, (C) in the presence of 500 µM E22L nanotube assemblies and (D) in the presence of 500 µM (K6pY)(E22L) nanotube assemblies.

![Figure 2](image2.png)

**Figure 2.** Atomic and electrostatic force micrographs of (A) (E22L) and (B) (K6pY)(E22L) nanotube assemblies. Topography (Left) and EFM amplitude (Right) micrographs of peptide nanotubes with a DC bias = +1 V. In the EFM amplitude micrographs, positively charged surfaces are white and negatively charged surfaces are dark.

![Figure 3](image3.png)

**Figure 3.** (A) TEM image of 500 µM (K6pY)(E22L) nanotube assemblies in the presence of 4.6 µM Histone H1-Alexa 488 conjugate. (B-D) A single slice of the two-photon fluorescence image excited at 780 nm of (B) 4.6 µM Alexa 488 labeled calf thymus histone H1 alone, (C) in the presence of 500 µM E22L nanotube assemblies and (D) in the presence of 500 µM (K6pY)(E22L) nanotube assemblies.

Histological titration calorimetry (ITC) theromograms show minimal heat change (Fig S9) between histone H1 and (E22L) assemblies. In contrast, the addition of histone to (K6pY)(E22L) nanotubes (Fig S10) fit to a one-site model with a large endothermic heat of binding (ΔH = 36.7 ± 5 kcal/mol) and -TΔS = -48 kcal/mol. This entropically driven association, with a Kₜ = 2.09 ± 0.3 x 10⁸ M⁻¹, is remarkably two orders higher than binding to calf-thymus DNA at the same temperature. Even more signif-
icantly, the saturation stoichiometry of $6.78 \pm 0.06 \times 10^{-3}$ binding sites per peptide translates into $147 \pm 13$ peptides per histone binding site.

A binding site of 147 peptides represents an area of $11.3 \times 5.2$ nm$^2$ on the nanotube surface, a large area for a 2.9 nm diameter globular protein with short unstructured N- and C- termini. To confirm the histone binding ITC values, fluorescence polarization (FP) is followed as (K6pY)(E22L) nanotubes are titrated into a fixed histone H1-Alexa 488 concentration (Fig. S1). Assuming non-cooperative association and 147 peptides per binding site, the $K_D$ of $4.8 \pm 2.9 \times 10^{-9}$ M, is identical to the $K_D$ determined by ITC ($4.78 \pm 0.58 \times 10^{-9}$ M). A recent evaluation of histone H1 binding to DNA identifies a binding site of 32 ± 1 base pairs$^{34}$, consistent with a histone length of 11 nm. With the 2.9 nm diameter of the globular domain, histone coverage area can be estimated as $11 \times 3 = 33$ nm$^2$, on the order of the surface area calculated for binding to the (K6pY)(E22L) nanotube surfaces and consistent with significant conformational plasticity upon binding.

The eukaryotic cell matrix presents a labyrinth of phospholipid membranes partitioning diverse compartments in a sea of ribonucleoproteins, phosphorylated metabolites and nucleic acid polymers. Such charged surfaces must all be navigated by the information processing cellular proteins that process nucleic acids. Histone H1 association with the DNA backbone$^{37}$ is important in gene-regulation,$^{28}$ chromatin condensation$^{39}$ and global control over chromatin remodeling activities.$^{30}$ However, H1 also permeates biological membranes and has been used to chaperone chimeric macromolecules across cell barriers,$^{3}$ highlighting the range of phosphorylated surfaces on which this protein functions.

The synthetic accessibility and morphological tractability of these patterned peptide surfaces$^{5,6}$ now open entirely new possibilities for studying protein associations. For example, given that histone H1 was the first nucleic acid binding protein to be identified as miss-localized in amyloid diseases$^{33}$ and the recent implications of accumulation of other information processing proteins in disease plaques,$^{34}$ this neurofibrillar tangle surrogate can be used for proteomic screening of AD tissues and other aberrant cellular assemblies, and exploration of seeding and co-assembly in disease etiology.$^4$

ASSOCIATED CONTENT

Supporting Information
Contains details of synthesis, assembly, CD, XRD, IR, AFM, NMR, ITC, and FP of histone binding. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
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