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Hydrophobic Nanoparticles Reduce the β -Sheet Content of SEVI Amyloid Fibrils and Inhibit SEVI-Enhanced HIV Infectivity

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S Supporting Information

ABSTRACT: Semen-derived enhancer of virus infection (SEVI) fibrils are naturally abundant amyloid aggregates found in semen that facilitate viral attachment and internalization of human immunodeficiency virus (HIV) in cells, thereby increasing the probability of infection. Mature SEVI fibrils are composed of aggregated peptides exhibiting high β -sheet secondary structural characteristics. Herein, we show that polymers containing hydrophobic side chains can interact with SEVI and reduce its β -sheet content by $\sim 45\%$ compared with the β -sheet content of SEVI in the presence of polymers with hydrophilic side chains, as estimated by polarization modulation-infrared reflectance absorption spectroscopy measurements. A nanoparticle (NP) formulation of this hydrophobic polymer reduced SEVI-mediated HIV infection in TMZ-bl cells by 60% compared with the control treatment. Although these NPs lacked specific amyloid-targeting groups, thus requiring high concentrations to observe biological activity, the use of hydrophobic interactions to alter the secondary structure of amyloids represents a useful approach to neutralizing the SEVI function. These results could, therefore, have general implications in the design of novel materials that can modify the activity of amyloids associated with a variety of other neurological and systemic diseases.

INTRODUCTION

Sexual contact is the most common mode of transmission of human immunodeficiency virus (HIV).^{1,2} Previous studies revealed that peptides found within semen are capable of aggregating into amyloid fibrils that bind strongly to HIV virions and cell membranes.^{3–9} For instance, semen-derived enhancer of virus infection (SEVI) is a naturally abundant amyloid found in semen and is composed of a peptide derived from the degradation of prostatic acid phosphatase, PAP (248–286).^{3,10,11} The binding of SEVI to viral and mammalian cell surfaces is believed to serve as a mediator for viral cell attachment and internalization, with the capability of increasing the rate of HIV infection through sexual contact by up to 400 000-fold.^{3,6,10,12} SEVI, therefore, represents a potentially important biological target for developing novel microbicide supplements to combat the spread of HIV.^{13–19}

Previous work in our laboratory has shown that high-affinity amyloid-binding small molecules, oligomers, and polymers can inhibit SEVI-mediated enhancement of HIV infectivity.^{20–22} These synthetic materials putatively create bioresistive coatings^{23,24} on the surface of SEVI fibrils that are capable of inhibiting the interaction of SEVI with various biologics (e.g., viral capsid proteins and cellular lipid membranes). These previous studies revealed that increased SEVI-binding affinity and the size of the amyloid-targeting agent resulted in increased efficacy for inhibiting SEVI-mediated HIV infection. Here, we explore a fundamentally new mechanism for inhibiting SEVI

function by examining whether hydrophobic nanoparticles (NPs) that lack amyloid-targeting moieties can disrupt the secondary structure of SEVI amyloid fibrils and whether this structural deformation is accompanied by the inhibition of SEVI-mediated enhancement of HIV infectivity (Figure 1).

Amyloids are well-known to adopt structures with significant β -sheet character,^{25–32} which generates highly stable structures that are often poorly soluble in aqueous environments and are resistant to enzymatic digestion.^{29,33,34} We and others have previously shown that amyloid fibrils can lose significant β -sheet content when they come in contact with hydrophobic surfaces.^{35–37} For instance, we showed that preformed aggregates of the Alzheimer-related amyloid- β ($A\beta$) peptides exhibited only $\sim 37\%$ β -sheet content when deposited on a hydrophobic polystyrene surface compared with the $\sim 60\%$ β -sheet content when deposited on a hydrophilic plasma-oxidized polystyrene surface.³⁵ Several other groups have also shown that hydrophobic materials can inhibit the fibrillization of $A\beta$, further supporting the possibility of using hydrophobic materials to disrupt the structural characteristics of $A\beta$ aggregates.^{36–40} Because there is evidence that the amyloid-form of PAP (248–286), and not the unaggregated peptide itself, enhances HIV infection,³ we hypothesized that disrupting

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Figure 1. Cartoon depicting the disruption of SEVI structure in the presence of hydrophobic NPs and concomitant reduction of SEVI-mediated enhancement of HIV infection.

the β -sheet content of SEVI using hydrophobic materials could lead to a new mechanism for inhibiting SEVI-mediated enhancement of HIV infectivity.

EXPERIMENTAL SECTION

Poly(octyl acrylate)-co-poly(hydroxyethylacrylamide) (POA-PHEAm, 4). Octyl acrylate (1, 381 mg, 2.1 mmol) and *N*-hydroxyethyl acrylamide (HEAm) (2, 26 mg, 0.21 mmol) were dissolved in dimethylformamide (DMF, 2.5 mL). Azobisisobutyronitrile (AIBN, 9 mg, 0.06 mmol) was added, and the reaction mixture was degassed by gently bubbling dry N_2 gas into the solution with stirring for 30 min. After degassing, the reaction vial was sealed under N_2 atmosphere and placed in an oil bath at 65 °C for 16 h. After cooling to room temperature (rt), the cloudy solution was transferred to a centrifugation vial and the polymerization vessel was rinsed twice with dichloromethane (DCM, 2 mL). The polymer was precipitated by the addition of methanol (MeOH, 40 mL), centrifuged to separate the solid polymer, and then the supernatant was decanted. The polymer was dissolved in DCM (5 mL) and again precipitated with MeOH (40 mL), followed by centrifugation and isolation of the solid. The final product was dried under vacuum to afford a colorless oil (83 mg, 20%). 1H NMR (400 MHz, $CDCl_3$): δ 4.00 (bs, 2H), 3.67 (bs, 2H), 2.27 (bs, 2H), 1.90 (bs, 1H), 1.60 (bs, 2H), 1.29 (bs, 10H), 0.88 (bs, 3H). Integration of the peaks at 4.00 and 3.67 ppm was used to determine the incorporation of monomer 1 (see the [Supporting Information](#) for details).

Poly(hexaaxanadecanoyl acrylate) (PHONA, 5). Hexaethylene glycol monomethyl ether acrylate (3, 284 mg, 0.81 mmol) and AIBN (3 mg, 0.02 mmol) were dissolved in butyl acetate (1 mL). The reaction mixture was degassed by gently bubbling dry N_2 gas with stirring for 30 min. After degassing, the reaction vial was sealed under N_2 atmosphere and placed in an oil bath at 65 °C for 16 h. After being cooled to rt, the cloudy solution was transferred to a centrifugation vial. The polymerization vessel was rinsed with DCM (2 $\times$ 2 mL) to facilitate complete transfer of the polymer. The polymer was precipitated by the addition of hexanes (40 mL), centrifuged to separate the polymer, and the supernatant was decanted. The polymer was dissolved in DCM (5 mL) and again precipitated with hexanes (40

mL). The product was dried under vacuum to afford a colorless oil (132 mg, 46%). 1H NMR (400 MHz, $CDCl_3$): δ 4.17 (2H, bs), 3.68 (22H, bm), 3.38 (3H, bs), 2.31 (1H, bs), 1.78 (2H, bs), 1.62 (1H, bs).

Nanoparticle (NP) Formulation. Hydrophobic polymer 4 (2 mg) was dissolved in 2 mL of tetrahydrofuran (THF), which was subsequently added dropwise to an equal volume of deionized (DI) water while stirring vigorously. After stirring for 30 min, the THF was removed in vacuo and the resultant NP solution (1 mg of total polymer per mL of DI water) was analyzed via dynamic light scattering. 1H NMR analysis of this same preparation of NPs using D_2O instead of DI water supported that this method efficiently removed THF from the NP suspension (see [Supporting Information](#) for the 1H NMR spectrum).

Polarization Modulation-Infrared Reflectance Absorption Spectroscopy (PM-IRRAS) Measurements. PM-IRRAS was conducted according to a previously described method.³⁵ Briefly, 10 μ L of a SEVI amyloid fibril sample (10 mg/mL total peptide concentration) was deposited onto a gold-plated glass slide coated with either polymer 4 or 5, ensuring that the material spread across the 1 cm height of the slide. The coated fragments were incubated in the dark for at least 6 h before the excess water was removed under a flow of N_2 . Each spectrum was recorded at 4 cm^{-1} resolution and is an average of at least 50 scans. Spectral analysis was conducted using the OMNIC Series software (ThermoFisher). The background infrared spectrum, the polymer-coated slide without SEVI incubation, was subtracted from the corresponding spectrum of SEVI amyloid fibrils incubated on the polymer coated slide. The spectra were then baseline-corrected between 1720 and 1580 cm^{-1} and smoothed at 9 cm^{-1} resolution. Second derivative analysis provided by the OMNIC Series software allowed for the resolution of conformational bands. The spectra were fitted between 1700 and 1600 cm^{-1} using least square iterative curve fitting with a mixture of Gaussian and Lorentzian line shapes. Secondary structures were quantified by calculating the areas of each component peak with respect to the original spectrum between 1700 and 1600 cm^{-1} .

Estimation of Amyloid Content Using a Thioflavin T (ThT) Fluorescence Assay. Fibril formation was monitored via ThT fluorescence as described previously.¹⁰ Briefly, fresh fibrils (10 mg/mL) were diluted 1:100 in a 25 μ M ThT working solution, and the fluorescence (Ex/Em 465/535, 20 nm bandwidth) was measured in an opaque 96-well black microtiter plate in a DTX 880 multimode plate reader.

Toxicity Assays. An Alamar Blue cell viability assay was performed in a 96-well plate format according to manufacturer's instructions (Invitrogen). Briefly, TZM-bl cells were plated at 7.5×10^3 cells/well and allowed to adhere overnight. The cells were then treated with either NP 4 or polymer 5 at 200 μ g/mL (final concentration) in growth media for 2 h. Fresh growth media were added along with the alamar blue reagent, and the fluorescence (Ex/Em 535/595) was measured using a DTX 880 multimode detector plate reader (Beckman Coulter).

Measurement of SEVI-Mediated Enhancement of HIV Infection in TZM-bl Cells. TZM-bl cells were plated at 7.5×10^3 cells/well and allowed to adhere overnight. Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was adjusted to pH 6 and then filter-sterilized through a 0.2 μ m syringe filter. NPs (final concentration 200 μ g/mL) were preincubated with or without SEVI fibrils (final concentration 15 μ g/mL) for 1 h at rt in the growth media. HIV_{III} virions (final p24 concentration of 85 ng/mL) were then added to the sample solutions and incubated for additional 15 min. Sample solutions were added to the cells in triplicates (100 μ L/well) and incubated for 2 h in a humidified 37 °C incubator to allow for infection, after which the initial inoculum was removed and fresh media of pH 7.5 were added. The cells were incubated for an additional 72 h at 37 °C. The cells were lysed by the addition of passive lysis buffer (50 μ L/well) (Promega) and incubation at 37 °C for 5 min. The lysates were clarified by centrifugation for 5 min at 300 $\times$ g in v-bottom 96-well plates. The clarified lysate (5 μ L) was combined with Quick Start Bradford reagent (BioRad) (200 μ L)

to measure the total cellular protein. The clarified lysate (25 μ L) was used to measure the luciferase activity by mixing with firefly substrate (Promega cat# E151A, 1:1 ratio) in opaque white plates. Bradford and luciferase were measured using a DTX 880 multimode detector plate reader (Beckman Coulter), and the luciferase values were normalized to the total cellular protein.

RESULTS AND DISCUSSION

Design, Synthesis, and Characterization of Polymeric Materials. To test the effect hydrophobic materials impose on SEVI-mediated HIV infection, we synthesized an acrylate-based polymer carrying octyl hydrocarbon side chains through coupling of octanol and acryloyl chloride under basic conditions followed by free-radical polymerization (Scheme 1). We found it necessary to incorporate a small percentage of a

Scheme 1. Synthesis of Polyacrylate-Based Hydrophobic (4) and Hydrophilic (5) Polymers^a

^aAbbreviations: triethylamine (TEA), dichloromethane (DCM), azobisisobutyronitrile (AIBN), dimethylformamide (DMF), potassium hydroxide (KOH), and methanol (MeOH).

hydrophilic monomer, HEAm, 2, into this polymer to prevent the uncontrolled aggregation and precipitation of this polymer from aqueous solution. Using a feed ratio of 1:10 of monomers 2 and 1, respectively, we generated POAPHEAm (4) polymers with a number average molecular weight (M_n) of 6.5 kDa and a polydispersity index (M_w/M_n) of 1.84 (Table 1 and Figure

Table 1. Polymer and NP Characterization

polymer	M_n (kDa)	M_w/M_n	%1	NP diameter (nm)	polydispersity
4	6.5	1.84	95	265 $\pm$ 37	0.16 $\pm$ 0.04
5	12.9	1.42	N/A	N/A	N/A

S1A). Polymer 4 was synthesized using uncontrolled free-radical polymerization with azobisisobutyronitrile (AIBN) and isolated by precipitation from methanol in ~20% overall yield. ¹H NMR analysis revealed that polymer 4 contained only 5% of HEAm monomer 2 (i.e., only half of the original feed ratio of monomer 2 was incorporated into the isolated polymer).

Because we observed that this hydrophobic material slowly aggregated in aqueous solution, we then generated NPs from this polymer to increase the homogeneity of the aggregate size and shape of the material (which we found significantly affects reproducibility in HIV infection assays). Although it was possible to increase the percentage of hydrophilic groups into these polymers by increasing the feed ratio of monomer 2 (data not shown), we found that incorporating higher percentages of hydrophilic monomers rendered these polymers too soluble in

aqueous solution to be formulated into NPs. Using polymer 4, we generated spherical NPs with an average diameter of 265 nm in solution and average polydispersity of 0.16, using a previously described solvent evaporation technique (Table 1 and Figure S2).^{22,41,42} Although this technique made it possible to reproducibly generate NPs with a similar size (± 37 nm over 5 preparations), other methods for NP preparation^{43–45} could potentially be explored to predictably generate a set of NPs with a range of different diameters.

As a control hydrophilic polymer, we also synthesized a previously reported PHONA (5) polymer containing 100% monomethyl hydroxyethylene glycol side chains in ~45% yield using the same free-radical polymerization procedure used to generate polymer 4 (Scheme 1, Table 1, and Figure S1B).²² We analyzed the effects of polymer 5 in subsequent SEVI structure and function assays without further changes to its formulation, as the high solubility of this polymer in aqueous solutions precluded the possibility for its conversion to stable NPs.

Quantification of β -Sheet and Amyloid Content in Mature SEVI Fibril Preparations.

To determine the effects of polymers 4 and 5 on the structure of SEVI amyloid fibrils, we used a previously described spin-coating procedure^{35,46} to create a thin film (~100 nm thick) of polymer on the surface of gold-plated microscope slides. We then used PM-IRRAS to analyze the β -sheet content of five preformed SEVI fibril samples after deposition on surfaces coated with either the hydrophobic or hydrophilic polymer. The capability to estimate β -sheet content from small quantities (10 μ g) of SEVI is a distinct advantage of PM-IRRAS compared with techniques such as circular dichroism, which would require impractical quantities of the aggregated peptide to determine secondary structural characteristics for this study.^{8,35,47,48} An examination of the amide I region between 1600 and 1700 cm^{-1} of the peptides (Figure 2A,B, black curves) in SEVI revealed that polymer 4 decreased the β -sheet content (red curves) of SEVI by an average of ~45% compared with the β -sheet content of SEVI deposited on a thin film of polymer 5 (Figure 2A–C, also see Figure S3 for representative PM-IRRAS spectra of all SEVI samples). Additionally, we found a good correlation (Pearson correlation coefficient (r) of 0.86)^{49,50} between the β -sheet contents of these SEVI fibril preparations (estimated from the PM-IRRAS measurements of SEVI deposited onto a film of hydrophilic polymer 5) and the fluorescence emission of ThT incubated with the SEVI fibril solution (Figure 2D); ThT fluorescence is typically used to estimate the abundance of amyloid fibrils in solution.^{11,51,52} In these experiments, aliquots from the same SEVI solutions were analyzed using either PM-IRRAS or ThT fluorescence measurements separately to eliminate any potential interference of ThT with the interaction of the polymers and SEVI. For ThT fluorescence measurements, no polymers or NPs were present in the SEVI samples. These studies support that polymer 4 significantly disrupts the β -sheet content of SEVI compared with hydrophilic polymer 5 and that the β -sheet content is a reasonable measure of the amyloid abundance of SEVI in solution.

To examine whether the β -sheet content of SEVI had an effect on the enhancement of HIV infectivity mediated by SEVI, we examined the same 5 preparations of SEVI used in the PM-IRRAS experiments in an HIV infection assay.^{12,20–22} Briefly, this assay uses TMZ-bl cells that express a luciferase reporter upon infection with HIV virions. Figure 3 shows the plots of the observed ThT fluorescence values or % β -sheet content of SEVI versus the enhancement of HIV infectivity

Figure 2. PM-IRRAS analysis of SEVI amyloid fibrils and the correlations between β -sheet content and the observed fluorescence of ThT in the presence of SEVI. The representative PM-IRRAS absorbance spectra (black lines) of preformed SEVI fibrils deposited on a gold-plated slide coated with hydrophilic polymer 5 (A) or hydrophobic polymer 4 (B). Iterative least-squares curve fitting^{58,59} of the amide I region reveals the approximate abundance of β -sheet (red curves), α -helix/unordered (green curves), β -turn (purple curves), and antiparallel β -sheet as well as possible side chain absorbances (blue curves) within each SEVI sample.⁶⁰ (C) Scatterplots showing the observed β -sheet content of five different preparations of SEVI fibrils (as estimated by PM-IRRAS measurements) deposited on surfaces coated with either hydrophilic (5) or hydrophobic (4) polymers. (D) Plot of the observed ThT fluorescence in five different SEVI preparations versus the estimated β -sheet content of the same SEVI fibrils when deposited on surfaces coated with hydrophilic polymer 5. The dashed lines indicate the 95% confidence interval; *** denotes P value <0.001 , as estimated by the paired t -test.

Figure 3. Plots of ThT fluorescence in SEVI samples or relative β -sheet content of SEVI vs the effects of SEVI on HIV infectivity (represented as fold enhancement compared with the level of HIV infection in the absence of SEVI). (A) Graph of ThT fluorescence vs fold enhancement of SEVI-mediated HIV infection for five different preparations of SEVI fibrils. (B) Graph of β -sheet content (as estimated by PM-IRRAS) and fold enhancement of SEVI-mediated HIV infection for the same five SEVI fibril samples used to generate the data in (A). The dashed lines indicate 95% confidence intervals.

induced by SEVI (shown as fold enhancement relative to the measured HIV infectivity in the absence of SEVI). This analysis reveals a good correlation between ThT fluorescence values ($r = 0.97$) or the percentage of β -sheet character of the SEVI ($r = 0.74$) sample versus the observed activity of SEVI for enhancing HIV infection. Taken together, these results further demonstrate that the capability of SEVI to enhance HIV infectivity is strongly dependent on its amyloid content, which can be approximated by its relative percentage of β -sheet character. We also conducted atomic force microscopy (AFM) experiments to characterize the interaction between NPs generated from polymer 4 and mature SEVI fibrils. Although these AFM studies support that the NPs indeed interact with the SEVI fibrils (see Figure S4), we did not find any clear evidence of gross morphological changes to the SEVI fibrils as a result of these interactions.

Hydrophobic NPs Inhibit SEVI-Mediated HIV Infection. To evaluate the effects of hydrophobic NPs composed of polymer 4 (NP 4) or hydrophilic polymer 5 on SEVI-mediated

HIV infection, we first examined the toxicity of these synthetic materials in TZM-bl cells and their effects on HIV infection in the absence of SEVI. Figure 4A shows that both NP 4 and polymer 5 were not toxic to these cells at concentrations equal to or below 400 $\mu\text{g/mL}$, which was twice the concentration of NP 4 or polymer 5 used in the infectivity assays. Although we did not observe any effect of NP 4 on HIV infection (in the absence of SEVI) at this concentration of NPs, we were surprised to observe that a 200 $\mu\text{g/mL}$ concentration of polymer 5 reduced HIV infection by $\sim 50\%$ compared with the treatment with buffer (vehicle) control (Figure 4B). Polymers containing polyethylene glycol groups (such as the side chain groups in polymer 5) are well-known to surround membrane surfaces and block interactions of various biologics with cells.^{53,54} Results from a viral cell attachment assay²⁰ confirmed that HIV virions exhibit a reduced capability to attach to cells in the presence of polymer 5 (Figure 4C), supporting that the effects of this polymer on HIV infection (in the absence of SEVI) was likely due to the known effects of similar hydrophilic

Figure 4. Graphs of the effects of NPs composed of polymer 4 (NP 4) and hydrophilic polymer 5 on cell viability, viral attachment, or HIV infection. (A) Effects of NP 4, polymer 5, and nonoxynol-9 (a commercial spermicide used here as a control) on cell viability. (B) Effects of NP 4 and hydrophilic polymer 5 on HIV infection in the absence of SEVI fibrils. (C) Cell attachment of HIV virions (as estimated by p24 concentration) in the presence or absence of either NP 4 or polymer 5. (D) Effect of NP 4 and 5 on SEVI-mediated HIV infection, expressed as fold change of HIV infection relative to cells treated with vehicle or polymers in the absence of SEVI fibrils. ($n = 5$, mean of means $\pm$ SEM). All luciferase values for the effects of particles were normalized to the total cellular protein as determined by the Bradford assay. The concentrations of the polymers were 200 $\mu\text{g/mL}$ for each assay. Statistical analysis using one-way analysis of variance with Dunn's post-test; ** denotes P value <0.01 , and * denotes P value <0.1 .

polymers on stabilization of cells^{53,54} and not likely due to any inherent antiviral activity. In contrast to polymer 5, NP 4 did not interfere with viral attachment in the absence of SEVI (Figure 4C).

In the presence of SEVI, a 200 $\mu\text{g/mL}$ concentration of the hydrophobic NPs (NP 4) significantly reduced SEVI-enhanced HIV infection, resulting in a 66% reduction in the enhancement of HIV infection by SEVI compared with SEVI-mediated HIV infection in the absence of the polymer (Figure 4D). A high concentration of NP 4 was necessary to observe the inhibition of SEVI-mediated infection because these particles do not contain any amyloid-targeting group that could increase their binding interaction with SEVI (which we omitted purposely to exclude mechanisms other than the disruption of SEVI β -sheet content that lead to inhibiting SEVI-enhanced HIV infections^{20–22}). For hydrophilic polymer 5, we did not observe any effect on SEVI-mediated enhancement of HIV infection compared with the effects of this polymer on HIV infection alone (i.e., in the absence of SEVI). Collectively, these results support the hypothesis that hydrophobic NPs can significantly affect the function of SEVI for enhancing HIV infection by reducing its β -sheet content.

CONCLUSIONS

In conclusion, we demonstrated a new mechanism for inhibiting SEVI-mediated HIV infection through the disruption of SEVI amyloid fibril secondary structure using hydrophobic NPs. PM-IRRAS measurements show that these hydrophobic polymeric materials reduce the β -sheet content of SEVI fibrils

compared with hydrophilic materials. A comparison of PM-IRRAS measurements and ThT fluorescence of different SEVI preparations revealed a strong correlation between the β -sheet content and amyloid characteristics (as estimated by ThT fluorescence) of SEVI fibrils. The β -sheet content and amyloid characteristics of SEVI fibrils also corresponded well with the capability of SEVI to enhance HIV infection. Although studies on the effects of hydrophobic materials and aggregated forms of Alzheimer-related amyloid- β ($A\beta$) peptides have been previously reported,^{35–39} the results reported here represent the first demonstration of using hydrophobic materials to alter the β -sheet content of SEVI amyloid fibrils and to affect their natural biological activity. Although the present study involves using hydrophobic interactions to inhibit the function of a potentially key amyloid mediator for sexual transmission of HIV, we anticipate that this fundamentally different approach to altering the function of amyloids through modulation of its secondary structure could be applied in the design of novel strategies to combat other amyloid-associated diseases. For instance, prion diseases, such as Creutzfeldt–Jakob disease, are characterized by infectious amyloid structures that are notoriously resistant to protease degradation.^{55–57} Current efforts are aimed at using hydrophobic materials to affect the secondary structure of prion amyloids and to increase their susceptibility to enzymatic processing, thereby decreasing the chance of infection and spread of the disease. Other efforts include the incorporation of amyloid-targeting agents into hydrophobic materials to improve their potency for disrupting

amyloid secondary structure and for inhibiting concomitant biological activity.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.langmuir.6b04295](https://doi.org/10.1021/acs.langmuir.6b04295).

Materials and procedures used in the synthesis and characterization of all monomers, PM-IRRAS measurement procedures, NP formulations, and in vitro assays (PDF)

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Author Contributions

[§]D.A.S. and J.M.C. contributed equally. D.A.S., J.M.C., L.B., M.C., C.P.K., S.D., and J.Y. designed the experiments and analyzed the data. D.A.S., J.M.C., L.B., M.C., Y.H.K., and G.L. executed the experiments. D.A.S. and J.Y. wrote the manuscript. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HIV, human immunodeficiency virus; SEVI, semen-derived enhancer of virus infection

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