F-actin binding regions on the androgen receptor and huntingtin increase aggregation and alter aggregate characteristics

Suzanne Angeli  
*University of California - San Francisco*

Jieya Shao  
*Washington University School of Medicine in St. Louis*

Marc I. Diamond  
*Washington University School of Medicine in St. Louis*

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**F-Actin Binding Regions on the Androgen Receptor and Huntingtin Increase Aggregation and Alter Aggregate Characteristics**

**Suzanne Angeli¹, Jieya Shao², Marc I. Diamond²**

1 Department of Neurology, University of California San Francisco, San Francisco, California, United States of America, 2 Department of Neurology, Washington University School of Medicine, St. Louis, Missouri, United States of America

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**Abstract**

Protein aggregation is associated with neurodegeneration. Polyglutamine expansion diseases such as spinobulbar muscular atrophy and Huntington disease feature proteins that are destabilized by an expanded polyglutamine tract in their N-termini. It has previously been reported that intracellular aggregation of these target proteins, the androgen receptor (AR) and huntingtin (Htt), is modulated by actin-regulatory pathways. Sequences that flank the polyglutamine tract of AR and Htt might influence protein aggregation and toxicity through protein-protein interactions, but this has not been studied in detail. Here we have evaluated an N-terminal 127 amino acid fragment of AR and Htt exon 1. The first 50 amino acids of ARN127 and the first 14 amino acids of Htt exon 1 mediate binding to filamentous actin in vitro. Deletion of these actin-binding regions renders the polyglutamine-expanded forms of ARN127 and Htt exon 1 less aggregation-prone, and increases the SDS-solubility of aggregates that do form. These regions thus appear to alter the aggregation frequency and type of polyglutamine-induced aggregation. These findings highlight the importance of flanking sequences in determining the propensity of unstable proteins to misfold.

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**Introduction**

Spinobulbar muscular atrophy (SBMA) and Huntington disease (HD) are devastating neurodegenerative diseases. SBMA is caused by an expanded CAG trinucleotide repeat that encodes a long polyglutamine tract in the androgen receptor (AR) [1], while HD is caused by an enlarged polyglutamine tract in the huntingtin (Htt) protein [2]. Proteolytic cleavage of AR and Htt appears to generate toxic, N-terminal fragments [3,4,5,6]. These are sufficient to recapitulate neurodegenerative phenotypes in vivo [7,8]. N-terminal fragments of expanded AR and Htt readily aggregate in vitro and in cell-culture models, thus making them useful in vitro terminal fragments of expanded AR and Htt readily aggregate, and in cell-culture models, thus making them useful in vitro.

Protein aggregation is associated with neurodegeneration. Polyglutamine expansion diseases such as spinobulbar muscular atrophy and Huntington disease feature proteins that are destabilized by an expanded polyglutamine tract in their N-termini. It has previously been reported that intracellular aggregation of these target proteins, the androgen receptor (AR) and huntingtin (Htt), is modulated by actin-regulatory pathways. Sequences that flank the polyglutamine tract of AR and Htt might influence protein aggregation and toxicity through protein-protein interactions, but this has not been studied in detail. Here we have evaluated an N-terminal 127 amino acid fragment of AR and Htt exon 1. The first 50 amino acids of ARN127 and the first 14 amino acids of Htt exon 1 mediate binding to filamentous actin in vitro. Deletion of these actin-binding regions renders the polyglutamine-expanded forms of ARN127 and Htt exon 1 less aggregation-prone, and increases the SDS-solubility of aggregates that do form. These regions thus appear to alter the aggregation frequency and type of polyglutamine-induced aggregation. These findings highlight the importance of flanking sequences in determining the propensity of unstable proteins to misfold.

**Results**

**ARN127 and Htt Exon 1 Bind F-Actin In Vitro**

To test for a direct interaction between AR, Htt, and F-actin in vitro, we used an F-actin co-sedimentation assay with recombinant GST-ARN127 or GST-Htt exon 1 containing 25 glutamine repeats (Fig. 1A). Coomassie staining confirmed protein purity (Fig. S1A,E). Protein preparations were precleared by ultracentrifugation to remove any pre-existing aggregates. 0.5 μM GST-ARN127(25) or 0.25 μM GST-Htt exon 1(25) was incubated with F-actin (4 μM) that had been pre-polymerized in vitro for 1 hour at 25°C. As a control, proteins were incubated with an equal

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* E-mail: diamondm@neuro.wustl.edu

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Indirect evidence implicates actin and/or actin-binding factors as an influence on polyglutamine-dependent aggregation of AR and Htt [10,21,22,23,24,25,26]. Y-27632, a rho-kinase (ROCK) inhibitor, reduces intracellular polyglutamine aggregation of Htt exon 1 and the N-terminal fragment of AR, termed ARN127 [10,25]. Y-27632 also attenuates Htt toxicity in Drosophila and improves motor function in mice [10,27]. Y-27632 blocks phosphorylation of profilin, an actin-binding protein that directly binds Htt, but not AR [28,29]. Profilin strongly inhibits aggregation of ARN127 and Htt exon 1 in cells [29], and decreases polyglutamine-mediated toxicity in Drosophila [22]. The anti-aggregation effects of profilin depend on both its polyproline binding activity (required to bind Htt), and its ability to bind G-actin (required to suppress both Htt and AR aggregation) [29]. In this study, we have identified regions of ARN127 and Htt exon 1 that bind filamentous actin (F-actin) in vitro, and investigate the effect of these regions on polyglutamine-dependent aggregation.
concentration of bovine serum albumin (BSA) instead of F-actin. After ultracentrifugation (100,000 x g), supernatant and pellet fractions were analyzed by western blot using antibody to GST. Western blot analysis was used rather than Coomassie stain because both proteins are very similar in size to G-actin (43 kDa). F-actin localized to the pellet fraction in all cases, as visualized by Coomassie stain (Fig. 1B). Both GST-ARN127(25) and GST-Htt exon 1 co-sedimented with F-actin while remaining soluble in its absence (Fig. 1B). GST alone did not co-sediment with F-actin. We determined the binding affinity of GST-ARN127(25) to F-actin by using a constant amount of protein (16 nM for ARN127, 150 nM for Htt exon 1) mixed with increasing amounts of F-actin (Fig. 1C, D). GST-ARN127(25) bound to F-actin with an approximate dissociation constant (Kd) of 1 μM (Fig. 1C), comparable to other actin-binding proteins [30].

Amino Acids 1-50 of ARN127 and 1-14 of Htt Exon 1 Mediate F-Actin Binding

We used deletion analysis to map the actin-binding regions of ARN127 and Htt exon 1 (Fig. 2A-C). Protein purity was assessed by Coomassie (Fig. S1A,C,E). Deletion of the polyglutamine domain of ARN127 and peptides C-terminal to the polyglutamine domain [AR1-57] had no appreciable effect on actin binding (Fig. 2B), while deleting the N-terminal peptides of AR (ARN127[25] and AR78-127) abolished binding (Fig. 2B). Constructs lacking the polyglutamine tract (ARN127[ΔQ]) or containing an expanded polyglutamine tract (ARN127[52]) bound actin equivalently to GST-ARN127(25) or GST-Htt exon 1 (25). Deletion of peptides C-terminal to the polyglutamine tract of Htt exon 1 (Htt15-92) had no effect on binding while deletion of the first 14 amino acids (Htt1-45) abolished binding (Fig. 2C,D). Thus, the first 50 amino acids (N50) of AR and the first 14 amino acids (N14) of Htt domain of ARN127 and peptides C-terminal to the polyglutamine tract [ARN127(ΔQ)] had no appreciable effect on actin binding (Fig. 2B), while deleting the N-terminal peptides of AR (ARN127[25] and AR78-127) abolished binding (Fig. 2B). Constructs lacking the polyglutamine tract (ARN127[ΔQ]) or containing an expanded polyglutamine tract (ARN127[52]) bound actin equivalently to GST-ARN127(25) or GST-Htt exon 1 (25). Deletion of peptides C-terminal to the polyglutamine tract of Htt exon 1 (Htt15-92) had no effect on binding while deletion of the first 14 amino acids (Htt1-45) abolished binding (Fig. 2C,D). Thus, the first 50 amino acids (N50) of AR and the first 14 amino acids (N14) of Htt
mediate interactions with F-actin in vivo. There is no apparent homology between these regions.

**Sensitivity to Actin-Regulatory Pathways Requires the First 50 Amino Acids of ARN127**

We have previously found that Y-27632, as well as a downstream ROCK target, profilin, inhibit ARN127 and Htt exon 1 aggregation [10,25,29]. Since both Y-27632 and profilin regulate actin assembly [31,32], we tested whether deletion of the actin-binding region of ARN127 would alter its response to their inhibitory activities. We employed fluorescence resonance energy transfer (FRET) to quantify these effects [10]. We were able to test AR using this assay, since deletion of the N14 region of Htt prevented significant FRET, possibly due to altered orientation of the CFP and YFP moieties. Expanded ARN127 and ARQC with 65 glutamines were tagged with fluorescence donor or acceptor tags, CFP or YFP, and co-transfected into HEK293 cells as previously described [10]. Y-27632 decreased ARN127(65)-CFP/YFP aggregation dose-dependently (Fig. 3A). Intriguingly, it increased the aggregation of ARQC(65)-CFP/YFP (Fig. 3A). Similarly, profilin dose-dependently reduced ARN127(65)-CFP/YFP aggregation, but was much less effective on ARQC(65)-CFP/YFP aggregation (Fig. 3B). Thus, the N50 region of AR mediates effects of these actin regulators on polyglutamine-mediated aggregation.

**N50 of ARN127 Affects Inclusion Type, Number, and Distribution**

To better characterize the behavior of expanded AR and Htt peptides within the cell, and to determine the influence of the actin-binding regions, we transfected expanded forms of each construct into C17.2 neural precursor cells, which were used for their ease of imaging. Identical results were obtained with HEK293 cells (data not shown). We observed clear differences between inclusions formed by ARN127(65)-YFP vs. ARQC(65)-YFP (Fig. 4A). ARN127(65)-YFP formed many different types of inclusions (single, multiple, nuclear, cytoplasmic), whereas ARQC(65)-YFP tended to form one perinuclear inclusion (Fig. 4A–F). After 48 h post-transfection, ARN127(65)-YFP expression resulted in multiple inclusions per cell in ~57% of cells vs. ~23% for ARQC(65)-YFP (Fig. 4G). ARN127(65)-YFP produced nuclear inclusions in ~19% of cells vs. ~5% of cells for ARQC(65)-YFP (Fig. 4H). These data are consistent with the idea that protein interactions mediated by the N50 domain of AR could alter the type of protein aggregates, as well as their subcellular localization. In contrast, we did not observe obvious inclusion differences between Htt exon 1 and HttQC. Htt exon 1/2-YFP and HttQC/2-YFP formed inclusions with similar visual characteristics (data not shown) as did Htt exon 1/97-H4 and HttQC/97-H4 (Fig. H-I-N), which contain a HIS-HA-HIS epitope tag [33] (Fig. 5B).

**N50 of ARN127 and N14 of Htt Exon 1 Promote Inclusion Formation**

To quantify the effect of N50 of ARN127 and N14 of Htt exon 1 on intracellular aggregation, we transiently transfected HEK293 cells with expanded AR constructs (ARN127(65)-YFP or ARQC(65)-YFP) or Htt constructs (Htt exon 1/2-YFP or HttQC/2-YFP), Htt exon 1/97-H4 or HttQC/97-H4, which contain a HIS-HA-HIS epitope tag and cultured the cells for 24 h (Fig. 5A–E). In each case, the QC proteins produced significantly fewer visible inclusions (Fig. 5C–E). Biochemical analysis of total cell lysates after 24 h in 2% SDS revealed that the soluble fractions of both ARQC(65)-YFP and HttQC/2-YFP were enriched, while the SDS-insoluble aggregates, were depleted (Fig. 5F–H). Thus, N50 of ARN127 and N14 of Htt exon 1 each promote the formation of inclusions and SDS-insoluble aggregates.

**N50 of ARN127 and N14 of Htt Exon 1 Promote SDS-Insoluble Aggregates**

The distinct effects of actin-binding regions on AR and Htt inclusion formation suggested that they might also change aggregate characteristics. Thus, we transfected HEK293 cells with the various constructs as above. 24 h post-transfection the cells were disrupted via syringe lysis and fractionated by centrifugation (15,000 x g) to compare soluble vs. insoluble species. Supernatant and pellet fractions were enriched, while the SDS-insoluble aggregates, were depleted (Fig. 5F–H). Thus, N50 of ARN127 and N14 of Htt exon 1 each promote the formation of inclusions and SDS-insoluble aggregates.
Figure 4. N50 of ARN127 influences inclusion type, number, and distribution. A–C, Confocal images of ARN127(65)-YFP inclusions in C17.2 cells at 48 h (60X). D–F, Confocal images of ARQC(65)-YFP inclusions in C17.2 cells at 48 h (60X). YFP-tagged proteins are in green, F-actin is stained with rhodamine-phalloidin (red) and DNA is stained with DAPI (blue). G, ARN127(65)-YFP more often forms multiple inclusions per cell than ARQC(65)-YFP (* = p<.002, Student’s t-test). H, ARN127(Q65)-YFP more often forms nuclear inclusions per cell than ARQC(65)-YFP (* = p<.002, Student’s t-test). Averages are from three separate transfections, counting at least 100 cells each. I–K, Confocal images of Htt exon 1 (97)-H4 in C17.2 cells at 48 h (60X). L–N, Confocal images of HttQC (97)-H4 in C17.2 cells at 48 h (60X). Immunofluorescence of HA-tagged Htt is in green, F-actin is stained with rhodamine-phalloidin (red) and DNA is stained with DAPI (blue). We did not observe significant differences in patterns of inclusion formation between the two Htt constructs.

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and insoluble proteins (Fig. 6A,B). The aggregates of ARQC(65)-YFP were much more readily dissociated in 2% SDS compared to ARN127(65)-YFP (Fig. 6A,B). Parallel experiments in C17.2 cells revealed similar results (Fig. 6A). We observed similar phenomena for Htt exon 1(97)-H4 vs. HttQC(97)-H4 (Fig. 6C). The pellet fraction containing HttQC(97)-H4 was significantly more SDS-insoluble aggregates than ARQC(65)-YFP and HttQC(72)-YFP. HEK293 cells were transiently transfected with the indicated constructs. After 24 h, cells were lysed in 2% SDS sample buffer, and subjected to SDS-PAGE and western blot with YFP antibody. Stack indicates the SDS-insoluble higher molecular weight aggregates trapped in the stacking gel; Sol indicates the SDS-soluble monomers. Tubulin indicates loading control. Deletion of amino terminal peptides reduced the overall proportion of SDS-insoluble material detected in the stacking gel. G, Quantification of relative insoluble to soluble fractions of ARN127 vs. ARQC (n = 3, * = p < 0.05, Student’s t-test). H, Quantification of relative insoluble to soluble fractions of Htt exon 1 vs. HttQC (n = 3, * = p < 0.05, Student’s t-test). Quantification by Image J.

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soluble (Fig. 6C,D). Thus flanking sequences of AR and Htt influence both the propensity for protein misfolding and the biochemical characteristics of the aggregates that result.

Discussion

In this study, we have focused on aggregation-prone fragments of AR (ARN127) and Htt (Htt exon 1), identifying amino acids 1–50 (N50) of ARN127 and 1–14 (N14) of Htt exon 1 as regions that influence polyglutamine-dependent aggregation. These regions were originally identified because they mediate binding to F-actin in vitro. However, we did not observe co-localization of ARN127 or Htt exon 1 with the F-actin cytoskeleton (data not shown). This could reflect lack of significant binding, or that intracellular binding to F-actin is transient. Deletion of the N50 region of ARN127 or the N14 region of Htt decreased the total number of inclusions formed, and the aggregates that did form were more SDS-soluble. Further, the N50 region of AR regulates the characteristics of inclusions formed and mediates its responsiveness to the anti-aggregation effects of Y-27632 and profilin. Thus, the flanking sequence of the polyglutamine region does not simply alter the propensity to form a polyglutamine aggregate, but can directly affect its aggregation rate and responses, the subcellular localization of the inclusions, and the biochemical characteristics of the aggregates.

The importance of flanking sequences on Htt exon 1 has previously been demonstrated: flanking sequences have been shown to directly regulate aggregation, toxicity, and the morphology of inclusions [13,34,35]. Given the importance of primary amino acid sequence on polyglutamine aggregation in vitro [12,14], we cannot exclude that the intracellular aggregation differences observed are solely due to altered intrinsic aggregation kinetics. However, this cannot explain all observable differences noted, such as altered distribution of polyglutamine inclusions. We have found that peptides containing either the first 50 amino acids of AR or the first 14 amino acids of Htt partition to Triton-insoluble cell fractions independent of their inherent solubility (Figs. S2, S3), suggesting that these regions could mediate protein interactions with macromolecular structures in the cytoplasm. Indeed, the first 17 amino acids of Htt have previously been implicated as a pro-aggregation-domain, a cytoplasm-targeting domain, and have been shown to bind mitochondria, the endoplasmic reticulum, and the Golgi apparatus [35,36]. This region is highly conserved across diverse species. Taken together, these studies imply that this region is likely to mediate protein interactions that play an important role in determining aggregation properties of the Htt peptide.

In this study, we find that sequences independent of the polyglutamine tract have profound effects on subcellular localization, detergent solubility, and inclusion formation of polyglutamine peptides. Given the apparent importance of these regions for protein interaction, this implies that modifiers of protein aggregation are likely to be found within the set of AR and Htt interacting proteins. Indeed, the recent finding that Htt-interacting proteins are enriched for genetic modifiers of toxicity is consistent with this idea [38]. Further study of AR and Htt binding proteins thus may reveal mechanisms that specify particular aggregation pathways and pathogenic features of each disease.

Materials and Methods

Constructs

Bacterial expression vectors for ARN127 Htt exon 1 and various derivatives were constructed via PCR amplification and subcloned in the pGEX4T1 backbone (Amersham Biosciences). Similarly, for mammalian expression vectors, ARQC and HttQC plasmids were constructed via PCR amplification and subcloned in the pGEX4T1 backbone (Amersham Biosciences). Variants of ARQC and HttQC were constructed by PCR amplification and subcloning into pEGFP-N1, pEYFP-N1 (Clontech), or pcDNA3 backbones. GST-Htt exon 1 constructs were originally obtained from Paul In this study, we find that sequences independent of the polyglutamine tract have profound effects on subcellular localization, detergent solubility, and inclusion formation of polyglutamine peptides. Given the apparent importance of these regions for protein interaction, this implies that modifiers of protein aggregation are likely to be found within the set of AR and Htt interacting proteins. Indeed, the recent finding that Htt-interacting proteins are enriched for genetic modifiers of toxicity is consistent with this idea [38]. Further study of AR and Htt binding proteins thus may reveal mechanisms that specify particular aggregation pathways and pathogenic features of each disease.

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Muchowski and mammalian pcDNA3-Htt-H4 constructs were originally obtained from Joani Stefansson.

**Cell Culture and Transfection**

HEK293 cells were plated at 250,000 cells per well in a 24-well dish and transfected with .3 μg total DNA with Lipofectamine Plus reagent (Invitrogen) according to the manufacturer's instructions. C-17.2 cells were plated at 100,000 cells per 24-well and transfected with .6 μg DNA with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were harvested at indicated times.

**FRET**

All FRET measurements were carried out 48 hours after transfection of HEK293 cells, read in 96-well cell-culture plates by a fluorescence plate reader (Tecan). HEK293 cells were transfected with .075 μg total ARN127CFP/YFP DNA in a 1:3 donor:acceptor ratio. Profilin DNA was co-transfected with AR constructs at concentrations of .075 μg, .15 μg, or .225 μg. pcDNA3 backbone vector was also co-transfected for a constant concentration for 24 h prior to FRET measurements[29].

**Confocal Microscopy**

All images were acquired on a C1sl confocal microscope (Nikon Instruments Inc.).

**Immunofluorescence**

C17.2 cells were mounted on polyornithine-coated glass coverslips 48 hours after transfection at a density of 20,000 cells per coverslip for 60X imaging. Cells were fixed in 4% parafomaldehyde, treated with .5% Triton, and blocked in 5% BSA for 1 hour. Coverslips were treated with HA antibody (1:500, Covance) overnight at 4°C, rinsed 4X and washed 3X with %1 TBS-Tween, and treated with donkey-anti-mouse Alexa-Fluor 488 (1:400, Molecular probes) for 1 h at 37°C. Coverslips were rinsed 4X and washed 2X with %1 TBS-Tween. F-actin was visualized with rhodamine-conjugated phallolidin (1:300, Molecular Probes) and the nucleus was stained with DAPI (Sigma). Coverslips were mounted with anti-fade mounting media (Invitrogen) and analyzed 24 h later.

**Inclusion Counting**

At least 100 HEK293 cells per transfection (3) were examined for total number of inclusions formed after 24 h. Approximately 100 C17.2 cells per transfection (3) were counted for inclusions. Cells were identified as having nuclear, cytoplasmic, or multiple inclusions, or a combination.

**Protein Purification**

GST-ARN127 plasmids were grown in E. Coli Rosetta 2 (DE3) competent cells (Novagen). Protein expression was induced with 1 mM (isopropyl β-d-thiogalactoside) IPTG for 3 h at 37°C. GST-Htt plasmids were grown in E. Coli SURE competent cells (Stratagene). Protein expression was induced with 1 mM IPTG for 3h at 30°C. Bacterial pellets were resuspended in resuspension buffer (PBS, .05% Tween, 1 mM PMSF, protease inhibitor tablet (Roche)) and lyed by sonication and 1% Triton. GST-tagged proteins were precipitated with glutathione sepharose (Amersham Biosciences) and eluted with equal volumes of elution buffer (50 mM Tris-HCl pH 8.0 10 μl 10 mM reduced glutathione). For cleavage of the GST-tag, 37.5 units of thrombin protease (Amersham Biosciences) was used for .5 ml of GST-bound glutathione sepharose 4B (Amersham Sepharose) at 4°C overnight. Thrombin was removed from cleaved AR with benzamidine sepharose 6B (Amersham Biosciences). Protein concentration was quantified via Bradford assay and Coomassie staining via Image J.

**Detergent Fractionation**

For unexpanded AR and Htt constructs, HEK293 cells were harvested 24 h post-transfection. Cell pellets were lysed in 130 μl cold lysis buffer (PBS, 1 % Triton, 5 mM EDTA, protease inhibitor cocktail (Roche)) and subjected to high-speed ultracentrifugation (100,000 x g) for 30 min at 4°C. Proteins were added to a pre-polymerized F-actin (4 nM) or a BSA control for 1 h on ice. Mixtures were ultracentrifuged for 30 min at 100,000 x g at 4°C. Supernatants and pellets were subjected to SDS-PAGE. F-actin pellets were visualized via Coomassie stain. GST-proteins were probed via western blots using GST antibody (Santa Cruz Bioscience).

**Antibodies**

Anti-rabbit N-20 antibody (1:1000, Santa Cruz) was used for the detection of cleaved AR products. Anti-rabbit GFP-antibody (Santa Cruz) or anti-mouse HA-antibody (Covance) was used for the detection of YFP-tagged or HA-tagged proteins at 1:2000 dilution for soluble proteins or 1:1000 dilution for insoluble higher-molecular weights. Anti-mouse MW7 was used to detect the C-terminus of Htt at 1:2000 dilution for soluble proteins or 1:1000 dilution for insoluble higher-molecular weights.

**In Vitro Solubility**

GST-ARN127(25), GST-ARNQ(32), GST-ARQC(36), GST-Htt exon 1(25), and GST-HttQC(25) were recombiantly purified and quantified as described above. 1.25 mg/ml of GST-AR or .75 mg/ml of GST-Htt purified proteins were ultracentrifuged (100,000 x g) and incubated at 37°C for 1 h to promote misfolding. For cleavage of the GST tag, 1.0 mg/ml of precleared GST-AR peptide were incubated with 1 NIH unit of thrombin (Invitrogen) overnight at 4°C. Proteins were ultracentrifuged and supernatant and pellet fractions were resuspended in SDS sample buffer.
buffer and subjected to SDS-PAGE. Proteins were analyzed via Coomassie stain.

**Supporting Information**

**Figure S1** Purity of Recombinantly Purified Proteins. A, Coomassie stain of GST-ARN127(25), GST-ARN127(DQ), and GST-ARN127(52). Lower molecular weight (LMW) contaminant is indicated B, Cleaved ARN127(25) co-sediments with F-actin but is soluble in the absence of F-actin. GST-tagged ARN127(25) was treated with thrombin to cleave off the GST tag. 1 μM of thrombin-cleaved ARN127(25) was mixed with 5 μM F-actin as in Figure 1. Western blot was used to detect the N-terminus of AR (N-20 antibody) or Coomassie for actin. C, Coomassie stain of GST and GST-AR fragments, AR1-57, AR50-127, and AR78-127, D, Cleaved AR1-57 cosediments with F-actin but is soluble in the absence of F-actin. GST-tagged AR1-57 was treated with thrombin to cleave off the GST tag. 1 μM of thrombin-cleaved AR1-57 was mixed with 5 μM F-actin. Western blot was used to detect the N-terminus of AR (N-20 antibody) or Coomassie stain for actin. E, Coomassie stain of GST-Htt exon 1(25), GST- Htt1-45, and GST- Htt1-92.

**Figure S2** N50 of ARN127 and N14 of Htt exon 1 mediate macromolecular interactions. A, Schematic of AR and Htt peptides fused to YFP. B, The N-terminus of AR mediates macromolecular interactions. HEK293 cells were transfected with YFP fusion proteins, lysed in 1% Triton, and subjected to ultracentrifugation (100,000 × g). Supernatant and pellet fractions were analyzed via SDS-PAGE. ARN127(25)-YFP (top band) and ARNQ(25)-YFP are present in the Triton-insoluble pellets of HEK293 cells, while ARQ(25)-YFP is not. Tubulin indicates loading control. C, Deletion of the N14 region of Htt exon 1(25) does not affect Triton solubility. Supernatant and pellet fractions were analyzed via western blot with MW7 antibody (detects the C-terminus of Htt). Tubulin indicates loading control. D, The first 17aa of Htt alone fused to YFP cause it to become insoluble in cell lysates. Blots are probed with YFP. Tubulin indicates loading control.

**Figure S3** Inherent Solubility of GST peptides. A, There are no differences in the inherent solubility of AR peptides. GST-tagged ARN127(25), ARNQ(32), and ARQC(36) are present in equal amounts in the pellet fractions after ultracentrifugation. B, Cleavage of AR peptides from GST does not mask drastic differences in inherent solubility. GST was cleaved from ARN127(25), ARNQ(32), and ARQC(36) with thrombin protease at 4°C overnight. Peptides were ultracentrifuged (100,000 × g) and supernatant and pellet fractions were analyzed via SDS-PAGE and Coomassie stain.

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**Author Contributions**

Conceived and designed the experiments: SA JS MID. Performed the experiments: SA. Analyzed the data: SA JS MID. Contributed reagents/materials/analysis tools: JS. Wrote the paper: SA MID.

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