Title

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Journal

Analytical chemistry, 90(24)

ISSN

0003-2700

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Publication Date

2018-12-01

DOI

10.1021/acs.analchem.8b03649

Peer reviewed

analytical chemistry

Article

Cite This: *Anal. Chem.* 2018, 90, 14316−14320

pubs.acs.org/ac

Identification of Patients with Preeclampsia by Measuring Fluorescence of an Amyloid-Binding Aryl Cyano Amide in Human Urine Samples

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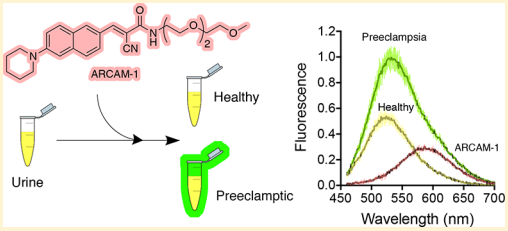
Supporting Information

ABSTRACT: Preeclampsia (PE) is a hypertensive disorder of pregnancy and one of the leading contributors to both maternal and perinatal morbidity and mortality. Reliable diagnostic parameters unique to the disorder that accurately define and diagnose PE are currently unavailable. Recent studies have revealed that PE is accompanied by the accumulation of amyloidogenic deposits in the placenta and the presence of congophilic amyloid-like protein aggregates in the urine. Here, we evaluate the capability of an amyloid-targeting aryl cyano amide (ARCAM-1) fluorophore to identify PE patients from analysis of urine samples. Our results reveal that this probe can distinguish patients with PE from gestationally healthy patients and patients suffering from non-PE hypertension, highlighting the potential for amyloid-targeting fluorophores to help identify PE patients during pregnancy.



Preeclampsia (PE) is an incurable gestational complication that affects 3−5% of pregnancies, accounts for 42% of maternal deaths, and is associated with 15% of all preterm births.[1−4] With clinical symptoms beginning between the second and third trimester, this gestational disorder manifests as a collection of systems complications including hypertension and, when severe, liver and renal dysfunction and thrombocytopenia.[1,2,5] Left untreated, PE can lead to eclampsia, threatening the mother with seizures and stroke.[2,6,7] The pathology of PE originates from the placenta rather than the fetus and, as a result, the most common treatment for preeclampsia is the removal of the placenta by delivery.[2,8,9] Because the underlying causes and molecular pathology of PE remain unclear, the heterogeneity in the observed symptoms complicate accurate diagnosis and treatment.

Currently, diagnosis of PE relies on the sudden onset of hypertension after 20 weeks of gestation from a normotensive patient and the appearance of elevated protein levels in urine (proteinuria).[2,10−12] However, in patients with pre-existing (pregestational) hypertension or renal disease, these symptoms do not specifically identify PE and often complicate accurate diagnoses.[2,3,13,14] The search for additional diagnostic parameters that define PE has led to methods that detect angiogenic and antiangiogenic growth factors in blood, which have shown encouraging results in clinical trials for identification of pregnant women with early onset PE.[5−7,9] Nevertheless, the diversity of symptoms continues to hinder the establishment of well-defined diagnostic parameters for PE.[3,4,14]

Recently, efforts have identified protein misfolding as a pathological feature associated with PE.[12,15,16] Buhimschi and co-workers reported the presence of amyloid-like aggregates in the placenta and urine of patients with preeclampsia using the conventional amyloid-binding dyes Congo Red (CR) and thioflavin-T (ThT).[12,15,17] The composition of these aggregates are heterogeneous and includes proteins such as SERPINA1, albumin, IgG K-light chain, ceruloplasmin, interferon-inducible protein 6−16, Amyloid Precursor Protein (APP) proteoforms, and β-amyloid (Aβ) peptides. Additionally, PE patients also exhibited increased mRNA levels for secretases that cleave APP to yield Aβ.[12] While APP is present in a healthy placenta, the amyloidogenic cleavage product Aβ is abundant in the placenta of patients with PE,[12] leading to the hypothesis that deposition of amyloid plaques may be central to the pathogenesis of preeclampsia.[12,14,16,18] However, it remains unclear how well the presence of aggregated misfolded proteins serves as a biomarker for PE in readily accessible biofluids such as urine.

We previously reported the structure and fluorescence properties of an amyloid-targeting aryl cyano amide molecular probe, ARCAM-1 (Figure 1A).[19] This dye, and its related family of fluorophores, exhibits a strong enhancement in fluorescence intensity and a hypsochromic shift (blueshift) of

Received: August 11, 2018
Accepted: November 16, 2018
Published: November 16, 2018

DOI: 10.1021/acs.analchem.8b03649
Anal. Chem. 2018, 90, 14316−14320

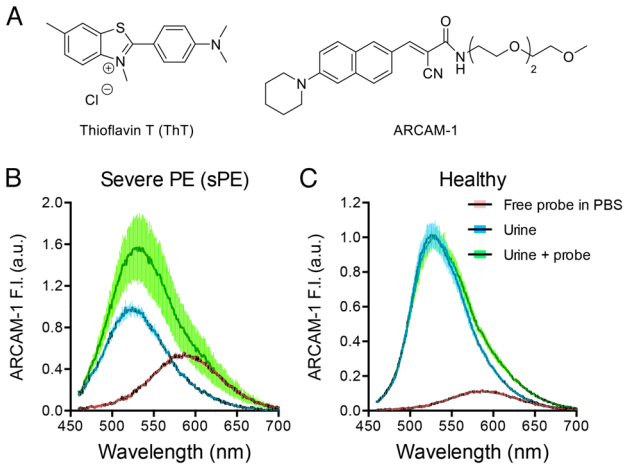


Figure 1. Spectral characteristics of urine from pregnant women in the presence of amyloid-targeting fluorescent probes. (A) Structures of amyloid-targeting thioflavin-T (ThT) and ARCAM-1. (B) The fluorescence intensity (F.I.) of ARCAM-1 in urine from a pregnant patient with superimposed severe preeclampsia (sPE) (green). For comparison, the fluorescence spectrum of the same urine sample (in the absence of ARCAM-1, blue) and of free ARCAM-1 in PBS (red) are also shown. (C) The fluorescence intensity (F.I.) of ARCAM-1 in urine from a healthy, pregnant patient (green). For comparison, the fluorescence spectrum of the same urine sample (in the absence of ARCAM-1, blue) and ARCAM-1 in PBS solution (red) are also shown. Spectra were normalized to the intrinsic fluorescence of urine.

fluorescence emission in the presence of Aβ aggregates in solution and in tissue.[20−23] We also showed that this probe binds to amyloids comprised of different proteins, suggesting that its binding properties are associated with its capability to recognize common secondary structural characteristics common to all amyloids (e.g., high β-sheet content).[21] We, therefore, hypothesized that this amyloid-specific probe could detect amyloid-like species present in urine specimens from PE patients, thereby distinguishing preeclamptic patients from healthy and non-PE hypertensive patients. Our previous studies suggest that ARCAM-1 is particularly well suited in this capacity because of its large increase of fluorescence intensity in the presence of amyloid aggregates in solution, and its demonstrated capability to bind to a larger range of amyloid species in solution compared to ThT.[19] Here, we report initial findings from a prospective study on the evaluation of fluorescence of ARCAM-1 in urine samples from 85 patients. We demonstrate that this probe could detect preeclamptic patients from gestationally healthy pregnant women as well as pregnant women suffering from non-PE-related hypertension by comparing differences in fluorescence intensity of ARCAM-1 added to urine samples.

EXPERIMENTAL SECTION

Study Participants and Urine Sample Collection. This blind prospective study was conducted following approval of the Institutional Review Board at UC San Diego. Informed consent was obtained from all participants. Maternal characteristics of patients in each group are tabulated in Supporting Information (SI) Table S-1. Briefly, we examined 109 urine samples from 85 patients, which were collected from patients who were admitted into the Labor and Delivery Unit or the Fetal Care and Genetics Center at UC San Diego Medical Center in San Diego, CA. Urine samples were obtained from spot collections, a classification for urine samples collected at a random time during the day.

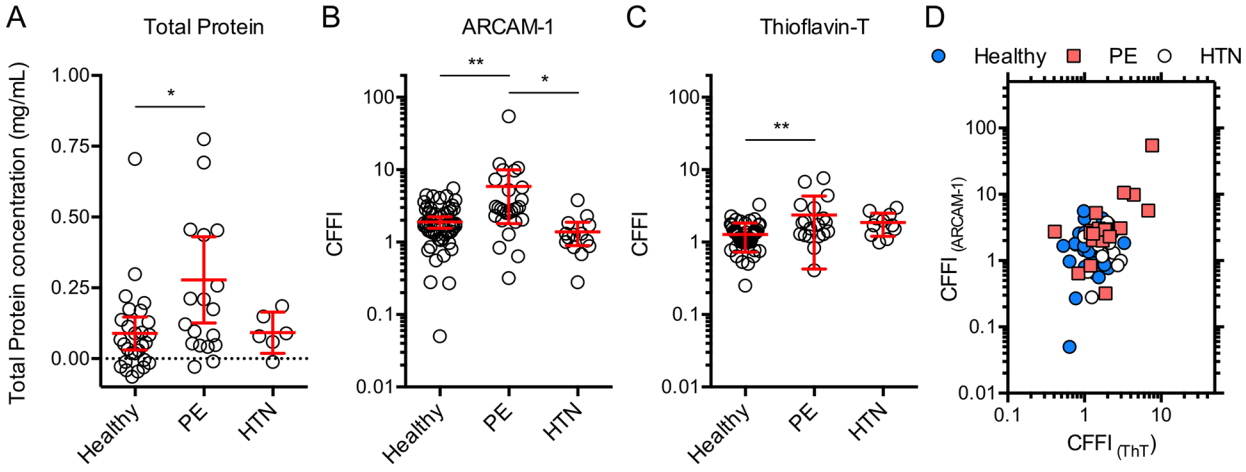
The collection criteria were (1) patients with normal pregnancies but at low risk for PE, (2) patients with normal pregnancies, but at high risk for PE (based on hypertension), 3) patients with symptoms of PE, and (4) patients with proteinuria, but no other symptoms of PE. All urine specimens were aliquoted prior to storage at −80 °C to prevent repeated freeze−thaw cycles. To avoid contamination with amniotic fluid, the urine from patients who experienced premature rupture of membranes were excluded from the study. The gestational age (GA) of women in this prospective study ranged from 11 to 40 weeks. The urine samples were centrifuged at 2000g and the supernatants retained for analyses. Adjudication of all patient disease status was performed following delivery by OB-Gyn physicians. Based on the adjudication results, we stratified the subjects into (a) healthy (n = 51), not diagnosed with hypertension or proteinuria; (b) preeclampsia (n = 26); and (c) non-PE hypertensive (HTN) (n = 8), which consisted of patients that exhibited symptoms of preeclampsia but were adjudicated with either chronic hypertension or gestational hypertension without preeclampsia.

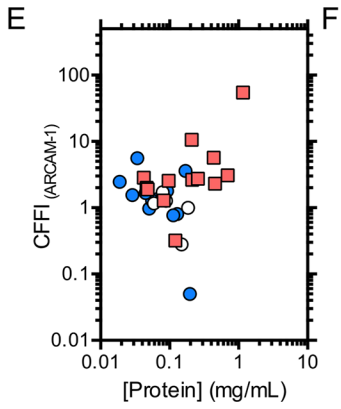
Quantification of Total Urinary Protein in Specimens. The Bradford protein quantification assay (Bio-Rad, 500−0006) was performed in accordance to the manufacturer's protocol. Briefly, a working solution of the Bradford dye concentrate was prepared by diluting one part of the Bio-Rad dye concentrate with four parts of Milli-Q water. A 200 μL aliquot of this working solution was incubated with 10 μL of 2 mg/mL standard BSA solution, or 10 μL of undiluted urine. Contents were vortexed and allowed to incubate at room temperature for 10 min. Samples were then vortexed again to prevent settling prior to analysis. Optical densities were measured at 595 nm on a SpectraMax 190 microplate reader (Molecular Devices). Analyses were performed in triplicate.

Analysis of ThT Fluorescence in Urine Samples. ThT fluorescence studies were conducted in accordance to a protocol described by Buhimschi et al.[12] Briefly, 70 μL of 114 μM ThT solution in phosphate buffered saline (PBS) was added to 30 μL of urine. The final concentration of ThT in the samples was 80 μM and the fluorescence of ThT in the samples measured within 5 min of adding the dye. Fluorescence spectra were recorded in triplicate using an EnSpire 2300 Multimode plate reader (PerkinElmer) with the excitation wavelength set at 444 nm, and emission spectra recorded from 450−600 nm. The urine autofluorescence was removed using a blank measurement without ThT. The corrected spectra were analyzed for dye binding by calculating the corrected fold increase of fluorescence intensity (CFFI), which we defined as the ratio between the fluorescence intensity at λ = 485 nm of ThT in urine versus in PBS alone.

Analysis of ARCAM-1 Fluorescence in Urine Samples. ARCAM-1 was synthesized as previously reported.[19] The fluorescence emission spectra of ARCAM-1 in urine were obtained using a modified amyloid-detection protocol for this class of molecules.[19,21] Briefly, 5 μL of 60 μM ARCAM-1 in dimethyl sulfoxide (DMSO) was added to 30 μL of urine in 65 μL of PBS to a final concentration of 3 μM. Samples were incubated at room temperature for 10 min prior to experiments. Fluorescence was measured using an EnSpire 2300 Multimode plate reader (PerkinElmer) with the excitation wavelength set at 450 nm, and emission spectra

DOI: 10.1021/acs.analchem.8b03649
Anal. Chem. 2018, 90, 14316−14320





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	Patients	Samples	Total protein content (mg/mL)	$CFFI_{ARCAM-1}$	$CFFI_{ThT}$
Gestationally healthy	51	64	0.089±0.028 (0.150)	1.896±0.167 (1.260)	1.279±0.082 (0.551)
Preeclamptic (PE)	26	31	0.278±0.072 (0.316)	5.862±1.973 (10.25)	2.371±0.446 (1.945)
Hypertensive (HTN)	8	14	0.092±0.028 (0.069)	1.388±0.228 (0.854)	1.857±0.196 (0.649)
Totals	85	109			

Figure 2. Comparison of detection methods for PE between different patient populations. (A) Total protein concentration in urine from PE patients is statistically higher than urine from healthy patients or non-PE patients with hypertension ($F(2,50) = 4.466$, $P = 0.0164$). (B) The CFFI of ARCAM-1 can distinguish PE patients from both gestationally healthy patients or hypertensive patients ($F(2,95) = 5.473$, $P = 0.0056$). (C) The CFFI of ThT can only distinguish PE patients from healthy patients ($F(2,72) = 6.956$, $P = 0.0017$), but not from the HTN patients. (D) Correlation analysis between the CFFIs of ThT and ARCAM-1 show a strong positive correlation for urine samples from PE patients ($r = 0.643$, $P = 0.003$) but not from gestationally healthy or HTN patients. (E) Correlation analysis between the CFFI of ARCAM-1 and total protein content shows a positive correlation only with urine samples from PE patients ($r = 0.611$, $P = 0.018$) but not from gestationally healthy or HTN patients. (F) Tabulated patient population examined in this blinded study. Statistical analyses were performed by one-way ANOVA, and the bars represent mean and standard error of the mean. Standard deviation for the total protein content and CFFI values are shown in parentheses in the table.

recorded from 460−700 nm. The urine autofluorescence was removed using a blank measurement without ARCAM-1. In order to calculate the corrected fold increase of fluorescence intensity (CFFI), we used the fluorescence emission λ_{max} of ARCAM-1 for each sample, as the dye is environmentally sensitive[20−22] (i.e., the observed λ_{max} varies with the protein composition of amyloid and amyloid-like aggregates). Therefore, the $CFFI_{ARCAM-1}$ was calculated as the ratio between the fluorescence at λ_{max} for each sample and the same λ_{max} of ARCAM-1 in PBS.

Statistical Analyses. Statistical analyses were calculated in GraphPad Prism 7.03 (San Diego, CA). The presence of outliers in the sample populations could not be examined using common methods of outlier detection (such as the Dixon Q-test, Grubb's test, and Rout test) because these statistical tests assume that the data conforms to a normal distribution. Instead, the Grubb's test for the outlier was applied after the data sets were fit to a Gaussian distribution using a logarithmic transformation.[24] A two-tailed p-value <0.05 was considered to be significant. While most patient urine samples were used for analysis of both the fluorescence of ARCAM-1 and ThT, some patient samples were included in analysis of only one fluorophore due to limited availability of urine samples. Population data is presented as mean and standard error of the mean (SEM) unless otherwise noted.

One-way analysis of variance (one-way ANOVA) was used to assess statistical significance of the total protein content versus the observed CFFI and the results are presented as means with standard error of the mean (SEM) values. Individual populations were compared with one another using Tukey's range test. Correlation analyses were performed using the nonparametric Spearman rank order correlation.

RESULTS AND DISCUSSION

To explore whether the amyloid-targeting fluorescent probe, ARCAM-1, could discriminate between healthy and PE patients, we first examined its fluorescence properties in a urine sample from a confirmed healthy patient versus a patient with severe PE (sPE). When incubated with the urine from the sPE patient, the emission profile of ARCAM-1 exhibited an 8-fold enhancement of fluorescence intensity and hypsochromic shift (i.e., blueshift) from 590 to 540 nm (Figure 1B), consistent with the previous findings showing that this class of compounds exhibits an enhancement of fluorescence emission in solutions containing amyloid-like aggregates.[20,21] In contrast, the urine from the healthy patient did not cause an

DOI: 10.1021/acs.analchem.8b03649
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enhancement of fluorescence of ARCAM-1 above the inherent sample autofluorescence (Figure 1C).

We next examined whether the observed differences in the fluorescence intensity of ARCAM-1 in urine from the PE and healthy patient could be a result of binding to amyloidogenic protein species or due to nonspecific interactions with, for example, serum proteins present in the PE patient urine sample. We surveyed the fluorescence of both ARCAM-1 and ThT (a common amyloid-binding fluorophore, Figure 1A) in the presence of increasing concentrations of immunoglobulin G (IgG), albumin from human serum (HSA), insulin, and aggregated Aβ(1−42) in PBS (SI Figure S-1). While a small degree of fluorescence enhancement for both probes was observed in the presence of these common serum proteins, we observed up to a 50- to 70-fold increase (p = 0.0095) in the fluorescent intensity for both fluorophores when they were exposed to 110 μM (0.5 mg/mL) aggregated β-amyloid (1−42) (SI Figure S-1). The significant enhancement of fluorescence intensity in the presence of amyloid aggregates compared to several representative serum proteins supports the hypothesis that general proteinuria (i.e., protein-containing urine that does not contain amyloid-like aggregates) does not cause significant increases in the fluorescence intensity of these fluorophores in urine samples.

We then assessed the ability of ARCAM-1 to distinguish PE patients from non-PE patients by examining urine samples from 85 pregnant women in a blinded, prospective study. Spot collections of urine were taken from each patient and analyzed for (1) proteinuria, (2) fluorescence of added ARCAM-1, and (3) fluorescence of added ThT (Figure 2A−C). Due to the limited number of patient and urine samples available, we separated patients by posthoc adjudications into only three groups consisting of gestationally healthy, nonpreeclamptic hypertensive (HTN), and preeclamptic (which included patients with superimposed hypertensive disorders[13,25]), but did not separate patients by severity of the disorders. In total, we examined 109 urine samples provided by the 85 patients (i.e., some patients provided multiple urine samples) (Figure 2F).

In gestationally healthy patients, the measured protein content in urine was typically undetectable or very low (0.089 ± 0.028 mg/mL) and was comparable to the average protein content of urine samples from the HTN population (0.092 ± 0.028 mg/mL). In the PE population, we found a 3-fold higher average concentration of total protein (0.278 ± 0.072 mg/mL, F(2,50) = 4.466, P = 0.0164, Figure 2A).

We next examined the fluorescence of ARCAM-1 when incubated with the urine samples from the three patient populations using a corrected fold increase of fluorescence intensity (CFFI), calculated as the ratio of fluorescence intensity at the observed λ_{max} for ARCAM-1 in urine versus the observed fluorescence intensity of the free probe in phosphate buffer. The ANOVA of the mean CFFI values using ARCAM-1 showed a significant 3.1-fold and 4.2-fold difference in average fluorescence signal between PE patients (CFFI = 5.862 ± 1.973) and the gestationally healthy (CFFI = 1.896 ± 0.167) or HTN (CFFI = 1.388 ± 0.228) populations, respectively (F(2,95) = 5.473, P = 0.0056, Figure 2B).

As a control, we also examined the fluorescence of ThT when incubated with the urine from the same patient populations. Comparison of mean patient CFFI values using ThT, where an emission wavelength of λ = 485 nm was used, showed a significant 1.8-fold difference in average signal between urine samples from PE patients (CFFI = 2.371 ± 0.446) versus the urine samples from the healthy (CFFI = 1.279 ± 0.082) populations. However, using ThT CFFI values was not able to distinguish PE patients from the HTN (CFFI = 1.857 ± 0.196) populations (F(2,72) = 6.956, P = 0.0017, Figure 2C). These results are consistent with recent reports of attempts to use ThT fluorescence to identify PE patients by inspection of amyloid content in urine and highlight a limitation of ThT as a potential diagnostic tool for PE—that is, the low enhancement of ThT fluorescence signal in PE positive samples.[12] Indeed, recent reports by Buhimschi have shown that ThT readily identifies only sPE patients from healthy or HTN patients,[17] suggesting that the fluorescence enhancement seen in urine samples from mild cases of PE may not be strong enough to dominate the signal from background fluorescence. In contrast to the results with ThT, the capability of ARCAM-1 to distinguish PE from a non-PE hypertensive population is particularly valuable as these two populations have overlapping clinical diagnostic criteria, which would otherwise obscure the identification of patients with PE.

Finally, we examined the potential utility of using fluorescent amyloid-binding probes as a stand-alone tool to detect PE in urine samples, without the need to examine secondary biometrics such as protein content (which was not able to readily distinguish PE from HTN patients). We compared the CFFI values of ARCAM-1 and ThT when the total patient sample population was split only into two groups: patients with PE and all non-PE patients. We found, as expected, that both probes could discriminate PE patients from all patients without PE (SI Figure S-2). However, ARCAM-1 showed a larger difference in CFFI values of PE versus non-PE patients compared to ThT, which we attributed to both its stronger emissive properties and the broad selectivity of the compound to a variety of amyloidogenic species (i.e., oligomers and fibrils).[19] Together, this strong sensitivity to PE supports that this probe has additional advantages as a screening tool for first-pass analyses of urine samples to identify PE.

In order to provide support that the results from fluorescence inspection of urine samples using ARCAM-1 was specific to PE patients, correlation analysis of the CFFI values of ARCAM-1 to the CFFI values of ThT in PE patient samples revealed a strong positive linear correlation (r = 0.643, P = 0.003), indicating that ARCAM-1 was likely detecting the same analytes (presumably amyloid-like species) as ThT in these patient samples (Figure 2D, red data). In contrast, a similar correlation analysis of CFFI values between the two probes in healthy or HTN patient samples showed no correlation (Figure 2D, blue and white data, respectively). We also found that higher protein content in the urine samples correlated with higher ARCAM-1 CFFI values in PE patient samples (r = 0.611, P = 0.018), whereas no correlation was seen in healthy or HTN patient samples (Figure 2E), suggesting that the increased fluorescence signal for ARCAM-1 in PE samples was due to detection of analytes specific to PE. Interestingly, we did not find a correlation between the ARCAM-1 CFFI values and the gestational age of the patients at the time of the spot collection, which we attribute, at least in part, to the variable nature of spot urine collections (SI Figure S-3).[26,27] These results suggest that amyloidogenic proteins are present in the urine of PE patients, and that the prevalence of amyloidogenic material is higher in PE patients with high protein content in their urine. Taken together, this data shows that while ThT, along with common

DOI: 10.1021/acs.analchem.8b03649
Anal. Chem. 2018, 90, 14316−14320

metrics to evaluate PE such as protein concentration, can distinguish between PE from healthy patients, these analyses cannot discriminate PE patients from non-PE patients with similar clinical symptoms. In contrast, ARCAM-1 is capable of distinguishing PE from gestationally healthy and non-PE hypertensive patients by simple inspection of fluorescence of this probe in urine samples.

CONCLUSIONS

We have presented an initial study for the capability of an amyloid-targeting fluorescent probe (ARCAM-1) to identify pregnant patients with preeclampsia through fluorescence inspection of urine samples. Importantly, while both ARCAM-1 and the conventional amyloid-targeting fluorophore thioflavin-T were capable of discriminating between urine samples from PE patients and urine samples from healthy patients, only ARCAM-1 was able to distinguish PE patients from patients with non-PE hypertensive disorders. The fluorescence signal from these probes appeared to correlate with urinary proteins that were specific to all PE patients. Collectively, these results support that amyloid-targeting fluorescent probes could enable a simple and accessible method for aiding in the diagnosis of PE in pregnant women.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b03649.

Additional patient population characteristics, protocol for preparation of aggregated β-amyloid(1−42) peptides, fluorescence data of molecular probes in the presence of serum proteins, and additional correlation analyses (PDF)

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Author Contributions

J.P.D., K.J.C., and J.Y. conceived the research. J.P.D., K.J.C., and S.W. designed and performed the experiments. J.P.D., K.J.C., L.L., M.P., and J.Y. analyzed and interpreted the results. J.P.D., K.J.C., and J.Y. wrote the manuscript. J.P.D. and K.J.C. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): JY is a founder of Amydis, Inc, who partially funded the work through a research gift.

ACKNOWLEDGMENTS

We thank Professor Nathan Gianneschi and Dr. Angela Blum for their instrumentation assistance for the spectroscopic studies. The cost for acquisition of urine samples was partially supported by a research gift from Amydis, Inc. Research reported in this publication was also partially supported by the National Institute on Aging of the National Institutes of Health under Award Number RF1AG062362.

REFERENCES

(1) Mol, B. W. J.; Roberts, C. T.; Thangaratinam, S.; Magee, L. A.; Groot, C. J. M. De; Hofmeyr, G. J. *Lancet* **2016**, *387*, 999−1011.
(2) Chaiworapongsa, T.; Chaemsaithong, P.; Yeo, L.; Romero, R. *Nat. Rev. Nephrol.* **2014**, *10*, 466−480.
(3) Stepan, H.; Hund, M.; Gencay, M.; Denk, B.; Dinkel, C.; Kaminski, W. E.; Wieloch, P.; Semus, B.; Meloth, T.; Dröge, L.; Verlohren, S.; Hund, M.; Gencay, M.; Denk, B.; Dinkel, C.; Kaminski, W. E. *Hypertens. Pregnancy* **2016**, *35* (3), 295−305.
(4) Verlohren, S.; Galindo, A.; Schlembach, D.; Zeisler, H.; Herraiz, I.; Moertl, M. G.; Pape, J.; Dudenhausen, J. W.; Denk, B.; Stepan, H. *Am. J. Obstet. Gynecol.* **2010**, *202* (2), 161.e1−161.e11.
(5) Rana, S.; Hacker, M. R.; Modest, A. M.; Salahuddin, S.; Lim, K.; Verlohren, S.; Perschel, F. H.; Karumanchi, S. A. *Hypertension* **2012**, *60* (2), 451−458.
(6) Rana, S.; Powe, C. E.; Salahuddin, S.; Verlohren, S.; Perschel, F. H.; Levine, R. J.; Lim, K.-H.; Wenger, J. B.; Thadhani, R.; Karumanchi, S. A. *Circulation* **2012**, *125* (7), 911−919.
(7) Maynard, S. E.; Karumanchi, S. A. *Semin. Nephrol.* **2011**, *31* (1), 33−46.
(8) Redman, C. W. *Placenta* **1991**, *12* (4), 301−308.
(9) Powe, C. E.; Levine, R. J.; Karumanchi, S. A. *Circulation* **2011**, *123* (24), 2856−2869.
(10) Sibai, B.; Dekker, G.; Kupferminc, M. *Lancet* **2005**, *365* (9461), 785−799.
(11) Noris, M.; Perico, N.; Remuzzi, G. *Nat. Clin. Pract. Nephrol.* **2005**, *1* (2), 98−114.
(12) Buhimschi, I. A.; Nayeri, U. A.; Zhao, G.; Shook, L. L.; Pensalfini, A.; Funai, E. F.; Bernstein, I. M.; Glabe, C. G.; Buhimschi, C. S. *Sci. Transl. Med.* **2014**, *6* (245), 245ra92−245ra92.
(13) Chan, P.; Brown, M.; Simpson, J. M.; Davis, G. *BJOG* **2005**, *112* (3), 280−285.
(14) Cheng, S.; Nakashima, A.; Sharma, S. *Am. J. Reprod. Immunol.* **2016**, *75*, 372−381.
(15) Buhimschi, C. S.; Baumbusch, M. A.; Dulay, A. T.; Lee, S.; Wehrum, M.; Zhao, G.; Bahtiyar, M. O.; Pettker, C. M.; Ali, U. A.; Funai, E. F.; Buhimschi, I. A. *BJOG* **2010**, *117* (3), 321−330.
(16) Kouza, M.; Banerji, A.; Kolinski, A.; Buhimschi, I. A.; Kloczkowski, A. *Phys. Chem. Chem. Phys.* **2017**, *19*, 2990−2999.
(17) Millen, K. R.; Buhimschi, C. S.; Zhao, G.; Rood, K. M.; Tabbah, S.; Buhimschi, I. A. *Hypertension* **2018**, *71* (6), 1185−1192.
(18) Mccarthy, F. P.; Adetoba, A.; Gill, C.; Bramham, K.; Bertolaccini, M.; Burton, G. J.; Girardi, G.; Cstat, P. T. S.; Poston, L.; Chappell, L. C. *Am. J. Obstet. Gynecol.* **2016**, *215* (4), 464.e1−464.e7.
(19) Guan, Y.; Cao, K. J.; Cantlon, A.; Elbel, K.; Theodorakis, E. A.; Walsh, D. M.; Yang, J.; Shah, J. V. *ACS Chem. Neurosci.* **2015**, *6* (9), 1503−1508.
(20) Chang, W. M.; Dakanali, M.; Capule, C. C.; Sigurdson, C. J.; Yang, J.; Theodorakis, E. A. *ACS Chem. Neurosci.* **2011**, *2* (5), 249−255.
(21) Cao, K.; Farashi, M.; Dakanali, M.; Chang, W. M.; Sigurdson, C. J.; Theodorakis, E. A.; Yang, J. *J. Am. Chem. Soc.* **2012**, *3*, 1−4.
(22) Cao, K. J.; Elbel, K. M.; Cifelli, J. L.; Cirera, J.; Sigurdson, C. J.; Paesani, F.; Theodorakis, E. A.; Yang, J. *Sci. Rep.* **2018**, *8*, 6950−6963.
(23) Cao, K. J.; Yang, J. *Chem. Commun.* **2018**, *54*, 9107−9118.
(24) Grubbs, F. E. *Technometrics* **1969**, *11* (1), 1−21.
(25) Valent, A. M.; Defranco, E. A.; Allison, A.; Salem, A.; Klarquist, L.; Gonzales, K.; Habli, M.; Adair, C. D.; Armistead, C.; Wang, Y.; Lewis, D.; Sibai, B. *Am. J. Obstet. Gynecol.* **2015**, *212* (4), 515.e1−515.e8.
(26) Cade, T. J.; Gilbert, S. A.; Polyakov, A.; Hotchin, A. *Aust. N. Z. J. Obstet. Gynaecol.* **2012**, *52* (2), 179−182.
(27) Hogan, M. C.; Reich, H. N.; Nelson, P. J.; Adler, S. G.; Cattran, D. C.; Appel, G. B.; Gipson, D. S.; Kretzler, M.; Troost, J. P.; Lieske, J. C. *Kidney Int.* **2016**, *90* (5), 1080−1089.

DOI: 10.1021/acs.analchem.8b03649
Anal. Chem. 2018, 90, 14316−14320