SUPPORTING INFORMATION

Cross-Seeding Enables Repurposing of Aurein Antimicrobial Peptide as a Promoter of Human Islet Amyloid Polypeptide (hIAPP)

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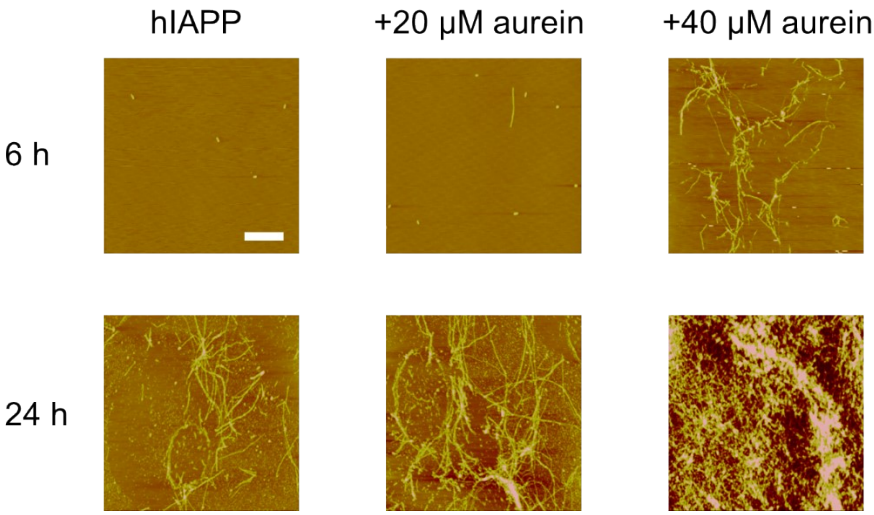

Figure S1. Cross-seeding of aurein with hIAPP to accelerate fibril formation. AFM images for pure hIAPP peptides (20 μM) in the absence and presence of different concentrations of aurein (20 μM and 40 μM) at 6 and 24 h. Scale bars are 1 μm.

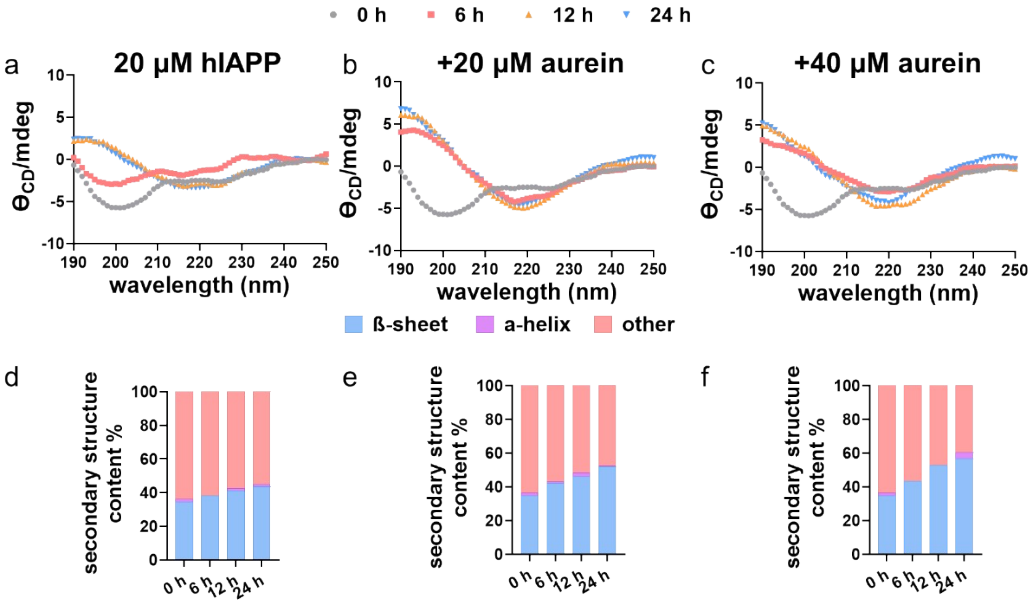

Figure S2. Cross-seeding of aurein with hIAPP to promote secondary structure transitions. (a-c) CD spectra and (d-f) the corresponding secondary structure contents for a 20 µM hIAPP in the (a, d) absence and presence of (b, e) 20 µM and (c, f) 40 µM of aurein during 24 h of incubation.

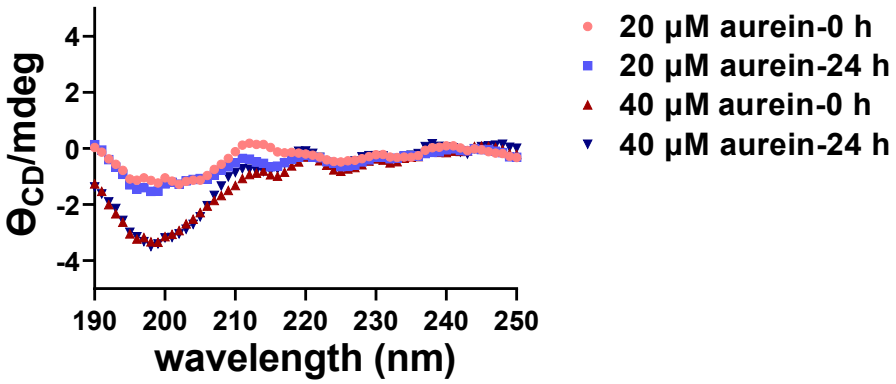

Figure S3. Time-dependent CD of aurein (20-40 μM) incubated at 37 ℃ for 0 and 24 h.

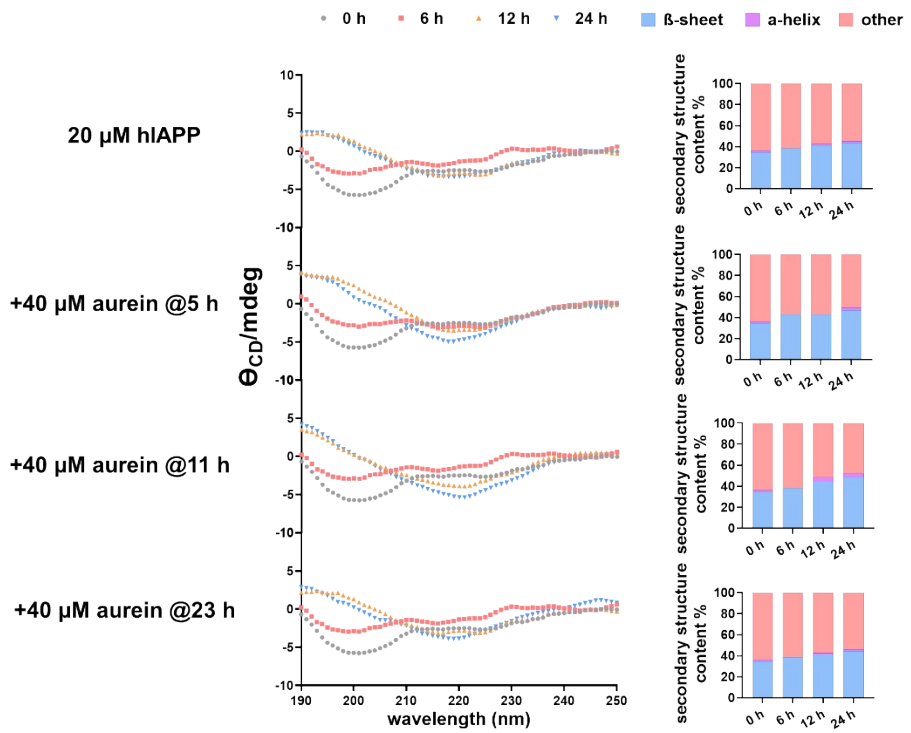

Figure S4. Cross-seeding of aurein with different hIAPP seeds to redirect amyloid formation pathways. Time-dependent CD spectra and corresponding secondary structure content for the cross-seeding of aurein (40 µM) with hIAPP (20 µM) seeds at different time points of 0, 5, 11, and 23 h.

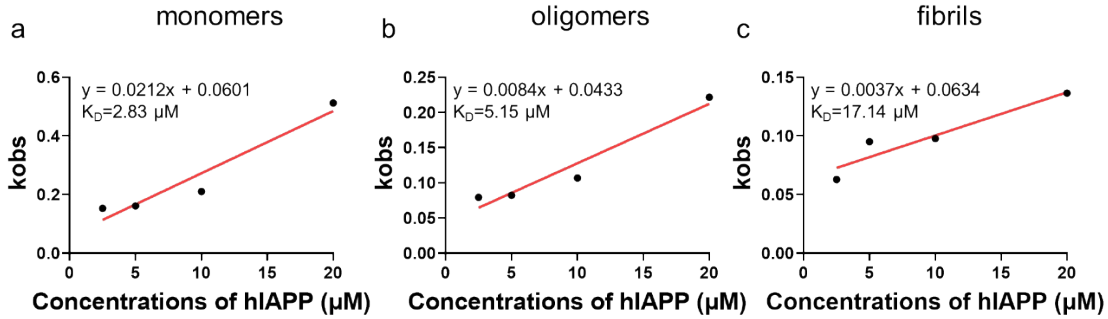

Figure S5. Binding constant (KD) of aurein with different hIAPP seeds of (a) monomers, (b) oligomers, and (c) fibrils calculated from SPR sensorgrams (**Fig. 3b**) by fitting observable binding constant kobs to amyloid concentrations.

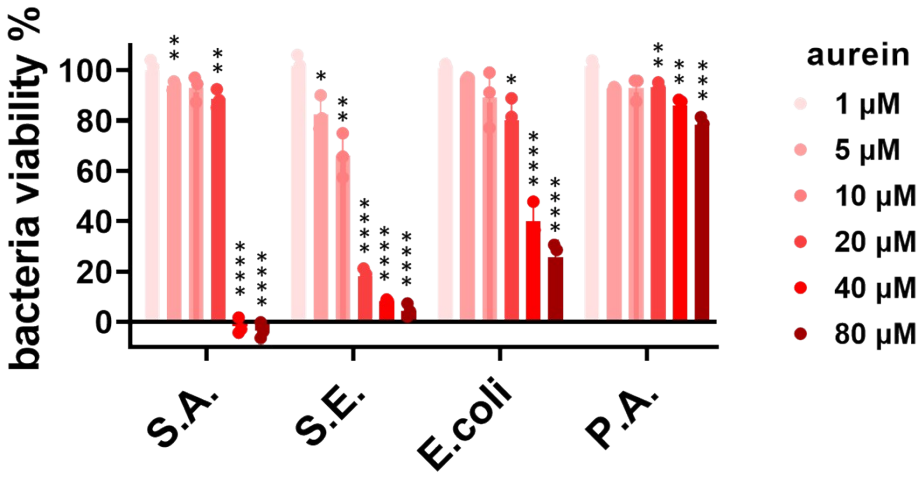

Figure S6. Dose-dependent anti-bacteria capacity of pure aurein (1-80 μM) against Gram-positive *S. A.* and (d) *S. E.* and Gram-negative *E. Coli* and (b) *P. A.* quantified by final bacterial density. All the Aurein-treated bacteria were normalized by untreated cells (positive control, 100% bacteria viability). All data represent mean ± s.d. of three independent experiments. Statistical analysis (n = 3) was performed for bacteria treated with aurein compared to untreated bacteria (i.e., bacterial viability=100%) (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$; ****, $p < 0.001$).

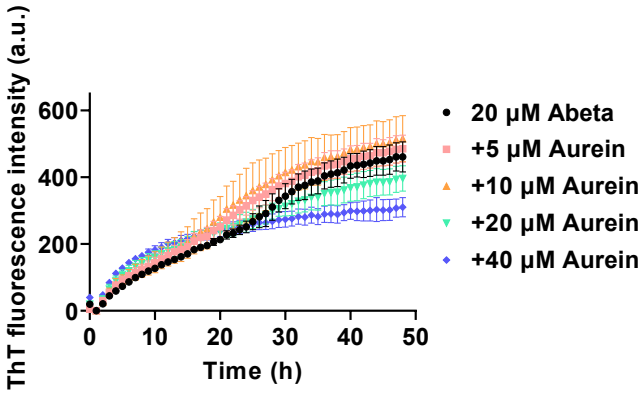

Figure S7. Dose-dependent promotion effect of aurein (10-40 μM) on Aebta (20 μM) aggregation by ThT fluorescence assays. Data represent mean ± standard error of triplicate measurements. (n=3).

Reference

(1) Chatterjee, S.; Khunti, K.; Davies, M. J. J. T. l. Type 2 diabetes. **2017,** *389* (10085), 2239.

(2) DeFronzo, R. A.; Ferrannini, E.; Groop, L.; Henry, R. R.; Herman, W. H.; Holst, J. J.; Hu, F. B.; Kahn, C. R.; Raz, I.; Shulman, G. I. J. N. r. D. p. Type 2 diabetes mellitus. **2015,** *1* (1), 1.

(3) Vijan, S. J. A. o. i. m. Type 2 diabetes. **2010,** *152* (5), ITC3.

(4) Wu, Y.; Ding, Y.; Tanaka, Y.; Zhang, W. J. I. j. o. m. s. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. **2014,** *11* (11), 1185.

(5) Blair, S. N. J. B. j. o. s. m. Physical inactivity: the biggest public health problem of the 21st century. **2009,** *43* (1), 1.

(6) Horta, B. L.; Loret de Mola, C.; Victora, C. G. J. A. p. Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: a systematic review and meta-analysis. **2015,** *104*, 30.

(7) Hillier, T. A.; Pedula, K. L. J. D. c. Characteristics of an adult population with newly diagnosed type 2 diabetes: the relation of obesity and age of onset. **2001,** *24* (9), 1522.

(8) De Pergola, G.; Triggiani, V.; Bartolomeo, N.; Nardecchia, A.; Angelo Giagulli, V.; Bruno, I.; Caccavo, D.; Silvestris, F. J. E., Metabolic; Targets, I. D.-D. Independent relationship of osteocalcin circulating levels with obesity, type 2 diabetes, hypertension, and HDL cholesterol. **2016,** *16* (4), 270.

(9) Jaikaran, E. T.; Clark, A. Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **2001,** *1537* (3), 179.

(10) Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A. A.; Ogurtsova, K. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice* **2019,** *157*, 107843.

(11) Zhang, M.; Hu, R.; Chen, H.; Chang, Y.; Gong, X.; Liu, F.; Zheng, J. Interfacial interaction and lateral association of cross-seeding assemblies between hIAPP and rIAPP oligomers. *Physical Chemistry Chemical Physics* **2015,** *17* (16), 10373.

(12) Kapurniotu, A. Amyloidogenicity and cytotoxicity of islet amyloid polypeptide. *Peptide Science: Original Research on Biomolecules* **2001,** *60* (6), 438.

(13) Selkoe, D. J. Folding proteins in fatal ways. *Nature* **2003,** *426* (6968), 900.

(14) Lorenzo, A.; Razzaboni, B.; Weir, G. C.; Yankner, B. A. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* **1994,** *368* (6473), 756.

(15) Weyer, C.; Maggs, D. G.; Young, A. A.; Kolterman, O. G. J. C. p. d. Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. **2001,** *7* (14), 1353.

(16) Guo, J.; Sun, W.; Li, L.; Liu, F.; Lu, W. Brazilin inhibits fibrillogenesis of human islet amyloid polypeptide, disassembles mature fibrils, and alleviates cytotoxicity. *RSC advances* **2017,** *7* (69), 43491.
(17) Ren, B.; Liu, Y.; Zhang, Y.; Zhang, M.; Sun, Y.; Liang, G.; Xu, J.; Zheng, J. J. J. o. M. C. B. Tanshinones inhibit hIAPP aggregation, disaggregate preformed hIAPP fibrils, and protect cultured cells. **2018,** *6* (1), 56.
(18) Zhang, Y.; Zhang, D.; Tang, Y.; Ren, B.; Liu, F.; Xu, L.; Chang, Y.; Zheng, J. J. M. A. Aromadendrin: a dual amyloid promoter to accelerate fibrillization and reduce cytotoxicity of both amyloid-β and hIAPP. **2020,** *1* (5), 1241.
(19) Sparks, S.; Liu, G.; Robbins, K. J.; Lazo, N. D. Curcumin modulates the self-assembly of the islet amyloid polypeptide by disassembling α-helix. *Biochemical and biophysical research communications* **2012,** *422* (4), 551.
(20) Franko, A.; Rodriguez Camargo, D. C.; Böddrich, A.; Garg, D.; Rodriguez Camargo, A.; Rathkolb, B.; Janik, D.; Aichler, M.; Feuchtinger, A.; Neff, F. J. S. r. Epigallocatechin gallate (EGCG) reduces the intensity of pancreatic amyloid fibrils in human islet amyloid polypeptide (hIAPP) transgenic mice. **2018,** *8* (1), 1116.
(21) Mishra, R.; Sellin, D.; Radovan, D.; Gohlke, A.; Winter, R. J. C. Inhibiting islet amyloid polypeptide fibril formation by the red wine compound resveratrol. **2009,** *10* (3), 445.
(22) Tang, Y.; Zhang, D.; Zhang, Y.; Liu, Y.; Gong, X.; Chang, Y.; Ren, B.; Zheng, J. J. A. A. B. M. Introduction and fundamentals of human islet amyloid polypeptide inhibitors. **2020,** *3* (12), 8286.
(23) Kusminski, C. M.; Mcternan, P. G.; Kumar, S. J. C. s. Role of resistin in obesity, insulin resistance and Type II diabetes. **2005,** *109* (3), 243.
(24) Hudish, L. I.; Reusch, J. E.; Sussel, L. J. T. J. o. c. i. β Cell dysfunction during progression of metabolic syndrome to type 2 diabetes. **2019,** *129* (10), 4001.
(25) Berbudi, A.; Rahmadika, N.; Tjahjadi, A. I.; Ruslami, R. J. C. d. r. Type 2 diabetes and its impact on the immune system. **2020,** *16* (5), 442.
(26) Oguntibeju, O. O. J. I. j. o. p., pathophysiology; pharmacology. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. **2019,** *11* (3), 45.
(27) Gonzalez, L. L.; Garrie, K.; Turner, M. D. J. B. e. B. A.-M. B. o. D. Type 2 diabetes–an autoinflammatory disease driven by metabolic stress. **2018,** *1864* (11), 3805.
(28) El Saghir, A.; Farrugia, G.; Vassallo, N. J. C.; Lipids, P. o. The human islet amyloid polypeptide in protein misfolding disorders: Mechanisms of aggregation and interaction with biomembranes. **2021,** *234*, 105010.
(29) Dupuis, N. F.; Wu, C.; Shea, J.-E.; Bowers, M. T. J. J. o. t. A. C. S. The amyloid formation mechanism in human IAPP: dimers have β-strand monomer− monomer interfaces. **2011,** *133* (19), 7240.
(30) Tang, Y.; Liu, Y.; Zhang, Y.; Zhang, D.; Gong, X.; Zheng, J. J. A. C. N. Repurposing a cardiovascular disease drug of cloridarol as hIAPP inhibitor. **2021,** *12* (8), 1419.

(31) Yang, G.; Wei, J.; Liu, P.; Zhang, Q.; Tian, Y.; Hou, G.; Meng, L.; Xin, Y.; Jiang, X. J. M. Role of the gut microbiota in type 2 diabetes and related diseases. **2021,** *117*, 154712.

(32) Albandar, J. M.; Rams, T. E. J. P. Global epidemiology of periodontal diseases: an overview. **2002,** *29* (1), 7.

(33) D'aiuto, F.; Sabbah, W.; Netuveli, G.; Donos, N.; Hingorani, A. D.; Deanfield, J.; Tsakos, G. J. T. J. o. C. E.; Metabolism. Association of the metabolic syndrome with severe periodontitis in a large US population-based survey. **2008,** *93* (10), 3989.

(34) Xie, K.; Xu, B.; Zhang, Y.; Chen, M.; Ji, Y.; Wang, J.; Huang, Z.; Zhou, K.; Xia, Y.; Tang, W. J. J. o. c. p. A multi-method evaluation of the effects of Inflammatory cytokines (IL-1β, IFN-γ, TNF-α) on pancreatic β-cells. **2018,** *233* (12), 9375.

(35) Wachlin, G.; Augstein, P.; Schröder, D.; Kuttler, B.; Klöting, I.; Heinke, P.; Schmidt, S. J. J. o. a. IL-1β, IFN-γ and TNF-α increase vulnerability of pancreatic beta cells to autoimmune destruction. **2003,** *20* (4), 303.

(36) Demmer, R.; Jacobs Jr, D.; Singh, R.; Zuk, A.; Rosenbaum, M.; Papapanou, P.; Desvarieux, M. J. J. o. d. r. Periodontal bacteria and prediabetes prevalence in ORIGINS: the oral infections, glucose intolerance, and insulin resistance study. **2015,** *94* (9_suppl), 201S.

(37) Demmer, R. T.; Breskin, A.; Rosenbaum, M.; Zuk, A.; LeDuc, C.; Leibel, R.; Paster, B.; Desvarieux, M.; Jacobs Jr, D. R.; Papapanou, P. N. J. J. o. c. p. The subgingival microbiome, systemic inflammation and insulin resistance: the oral infections, glucose intolerance and insulin resistance study. **2017,** *44* (3), 255.

(38) Hjelmesaeth, J.; Asberg, A.; Muller, F.; Hartmann, A.; Jenssen, T. J. C. d. r. New-onset posttransplantation diabetes mellitus: insulin resistance or insulinopenia? Impact of immunosuppressive drugs, cytomegalovirus and hepatitis C virus infection. **2005,** *1* (1), 1.

(39) Hjelmesaeth, J.; Sagedal, S.; Hartmann, A.; Rollag, H.; Egeland, T.; Hagen, M.; Nordal, K.; Jenssen, T. J. D. Asymptomatic cytomegalovirus infection is associated with increased risk of new-onset diabetes mellitus and impaired insulin release after renal transplantation. **2004,** *47*, 1550.

(40) Westwell-Roper, C.; Dai, D. L.; Soukhatcheva, G.; Potter, K. J.; van Rooijen, N.; Ehses, J. A.; Verchere, C. B. J. T. j. o. i. IL-1 blockade attenuates islet amyloid polypeptide-induced proinflammatory cytokine release and pancreatic islet graft dysfunction. **2011,** *187* (5), 2755.

(41) Masters, S. L.; Dunne, A.; Subramanian, S. L.; Hull, R. L.; Tannahill, G. M.; Sharp, F. A.; Becker, C.; Franchi, L.; Yoshihara, E.; Chen, Z. J. N. i. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. **2010,** *11* (10), 897.

(42) Farrim, M. I. d. J. D., 2021.

(43) Wang, C.; Ma, Y.-H.; Han, X.; Lu, X. J. L. Re-Examining Interaction between Antimicrobial Peptide Aurein and Model Cell Membranes via SFG. **2022**.

(44) Fernandez, D. I.; Le Brun, A. P.; Whitwell, T. C.; Sani, M.-A.; James, M.; Separovic, F. J. P. C. C. P. The antimicrobial peptide Aurein disrupts model membranes via the carpet mechanism. **2012,** *14* (45), 15739.

(45) Wang, L.; Liu, Q.; Chen, J.-C.; Cui, Y.-X.; Zhou, B.; Chen, Y.-X.; Zhao, Y.-F.; Li, Y.-M. J. B. c. Antimicrobial activity of human islet amyloid polypeptides: an insight into amyloid peptides' connection with antimicrobial peptides. **2012,** *393* (7), 641.

(46) Micsonai, A.; Wien, F.; Kernya, L.; Lee, Y.-H.; Goto, Y.; Réfrégiers, M.; Kardos, J. Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy. *Proceedings of the National Academy of Sciences* **2015,** *112* (24), E3095.

(47) Li, Z.; Narouz, M. R.; Munro, K.; Hao, B.; Crudden, C. M.; Horton, J. H.; Hao, H. Carboxymethylated dextran-modified n-heterocyclic carbene self-assembled monolayers on gold for use in surface plasmon resonance biosensing. *ACS applied materials & interfaces* **2017,** *9* (45), 39223.