Electronic Supplementary Information

Supramolecular Photochirogenesis with Functional Amyloid Superstructures: Product Chirality Switching by Chiral Variants of Insulin Fibrils upon Enantiodifferentiating Photocyclodimerization of 2-Anthracenecarboxylate

Masaki Nishijima,[a] Hidekazu Tanaka,[b] Cheng Yang,[b,c] Gaku Fukuhara,[b] Tadashi Mori,[b] Viktoria Babenko,[d] Wojciech Dzwolak,[d]* and Yoshihisa Inoue[b]*

[a] *Office for University-Industry Collaboration and* [b] *Department of Applied Chemistry, Osaka University, Yamada-oka, Suita 565-0871, Japan*

[c] *College of Chemistry, Sichuan University, Chengdu 610064, P. R. China.*

[d] *Department of Chemistry, University of Warsaw, Pasteur 1, 02-093 Warsaw, Poland*

Email: wdzwolak@chem.uw.edu.pl, inoue@chem.eng.osaka-u.ac.jp

Table of Contents

Experimental

General. Bovine insulin and 2-anthracenecarboxylic acid were purchased from Sigma and TCI, respectively, and used without further purification. Na_2HPO_4, KH_2PO_4, trifluoroacetic acid were also used as received. Acetonitrile for product extraction and HPLC analyses was distilled prior to use and deionized water was used throughout the work.

UV-vis and CD spectra were recorded at 25 °C on JASCO V-560 or V-550 spectrophotometer and a JASCO J-820 spectropolarimeter, respectively, both of which were equipped with an ETC505T temperature controller.

Phosphate buffer solution (PBS) of 33 mM concentration was prepared by dissolving Na_2HPO_4 and KH_2PO_4 (2:1 ratio) in deionized water, and then adjusted to pH 7.0 by adding a KH_2PO_4 solution with pH monitoring. All insulin fibril (IF) solutions were buffered at pH 7.0 with PBS containing 0.1 M NaCl. A 5.0 mM AC stock solution was prepared by dissolving an appropriate amount of AC in aqueous 10 mM NaOH solution.

Preparation of (+)-, (–)- and (0)-insulin IFs. Both (+)- and (–)-IF amyloid fibrils were obtained by the so-called "vortex-induced chiral bifurcation" process described earlier.[1] Typically, ten Eppendorf tubes (1.5 mL volume), each containing aqueous 0.1 M NaCl solution (0.6 mL) of 1 wt % bovine insulin at pH 1.9, were vortexed for 48 h at 45 °C in an Eppendorf Thermomixer Comfort accessory at 1400 rpm. During this incubation routine, the insulin samples randomly converted either to (+)- or (–)-IF type of amyloid, which was determined afterwards through induced circular dichroism (ICD) measured after staining the fibrils with Thioflavin T (ThT), as described in our earlier work.[1]

The amounts of (+)-, and (-)-IFs used for photochirogenesis were identical in terms of total insulin concentration. Both types of fibrils were formed through the 45 ºC chiral bifurcation protocol which strongly favors IFs with extreme "ee" of either (+) or (-) type.[1,2] Aliquots of (+)- and (-)-IF thus obtained were subjected to the CD spectral examination with added ThT to determine the sign of IF from the circular dichroism induced to ThT. Comparison with the IF samples formed at lower and higher temperatures (where only (+)- and (-)-IFs are formed in a deterministic fashion) showed that IFs formed through the chiral bifurcation were at least 90 % enantiomerically pure.

(0)-IF amyloid samples were prepared through a quiescent 48 h-long incubation at 65 ^{o}C of the same stock solution of native insulin in 0.1 M NaCl at pH 1.9. IF samples formed upon quiescent incubation gave flat ICD spectra after staining with ThT.[1]

Spectral titrations of (+)-, (–)-, and (0)-IF with AC. UV-vis and CD spectral titrations of (+)-, (–)-, and (0)-IF with AC were performed in PBS (pH 7.0) containing 0.1 M NaCl at 25 °C to give the results shown in Figure S1. The (+)- and (–)-IF solutions (12 μM in insulin monomer) were not totally transparent and hence the titrations were run in a thin cell of 1 mm optical path, which allowed us to add AC of up to 5 equivalents (60 μM), while the (0)-IF solution was less opaque and the titration was run in a regular cell of 10 mm optical path, allowing AC addition of up to 1.6 equivalents (19.6 μM), due to the saturation of UV absorption at the peak top.

Regardless of the IFs used, the UV absorbance at the ^1B_b band (258 nm) of AC was strictly proportional to the concentration of AC (see the insets of Figures S1d-f), indicating that the UV-vis spectrum is not sensitive to the complexation of IF with AC. Interestingly, the CD spectra displayed significant ellipticity changes (θ ~ 10-15 mdeg) upon addition of AC to the solutions of (+)- and (–)-IF, inducing a positive and negative exciton couplet at the ^1B_b band (ca. 260 nm) and negative and positive Cotton effects at the ^1C_b band (ca. 220 nm), respectively (Figures S1d-f, top).

In contrast, practically no CD spectral change of θ >1 mdeg was induced upon addition of AC to a (0)-IF solution, as was the case with ThT added to (0)-IF.[2] However, this does not immediately mean the absence of AC-IF interactions but rather indicates that the net chiral bias of AC-binding moieties of (0)-IF differs in chiral environment from that of (+)- and (-)-IF fibrils, since the AC-(0)-IF mixture gave the photocyclodimers in a HH/HT ratio of 1.0 (Table 1 in the main text), which is higher than that for free AC (HH/HT = 0.4), and also in significant ee's for both **2** and **3** upon irradiation.

UV-vis and CD spectral titration of (+)-, (–)-, and (0)-IFs with AC

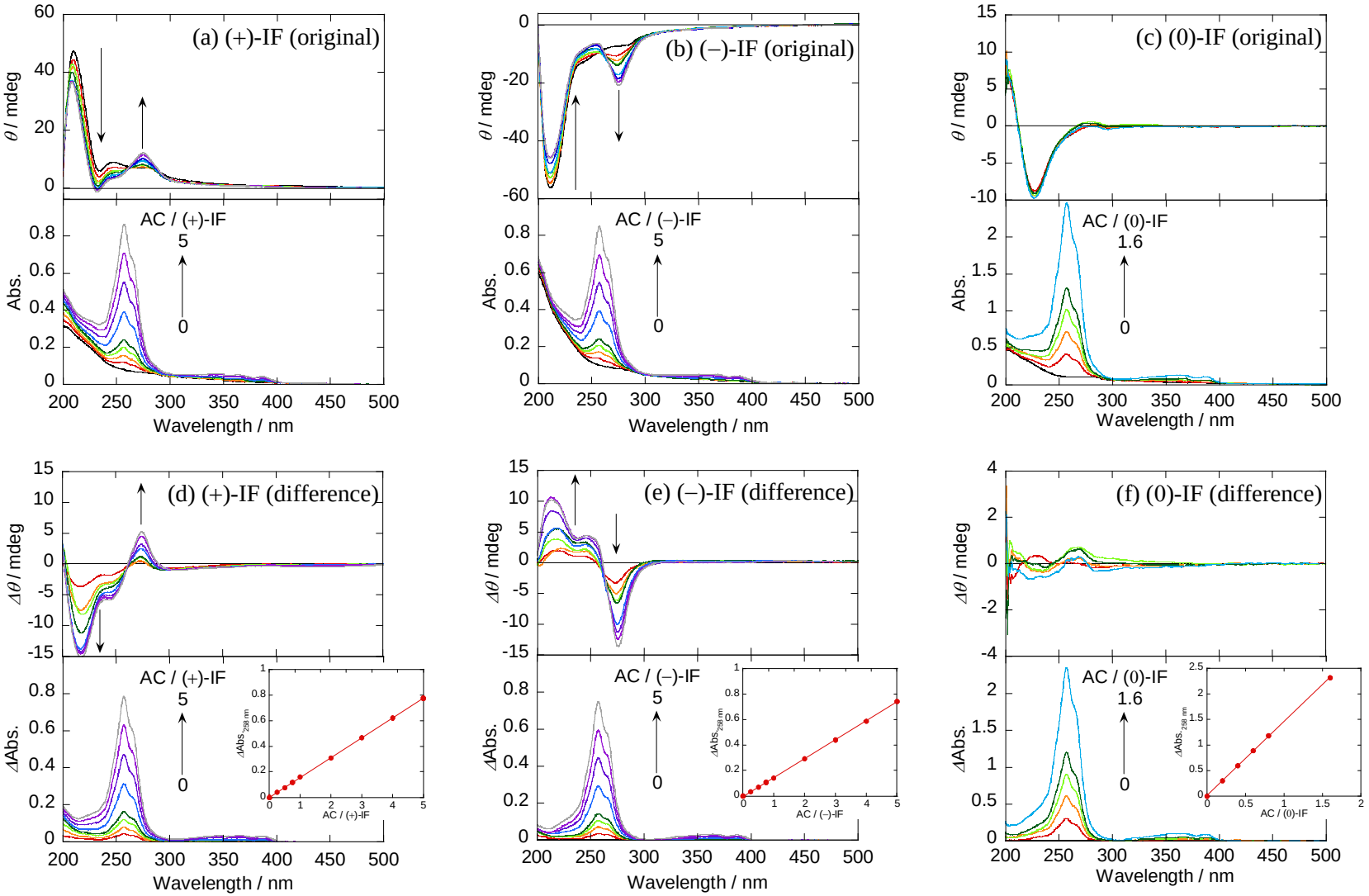

Fig. S1 *Upper panels (a)-(c)*: UV-vis (bottom) and CD (top) spectral changes upon addition of AC to (a) (+)-, (b) (–)-, and (c) (0)-IF (12 μM in insulin monomer) in PBS (pH 7.0, containing 0.1 M NaCl) at 25 °C. *Lower panels (d)-(f)*: Net UV-vis and CD changes induced by the addition of AC; Inset: the absorbance at 258 nm as a function of AC concentration.

UV-vis and CD spectral titration of native insulin with AC. As a control experiment, the native insulin used for the preparation of IFs was also subjected to the UV-vis and CD spectral titration with AC under similar conditions to show no appreciable change in CD and a linear increase of the absorbance with AC concentration (Figure S2).

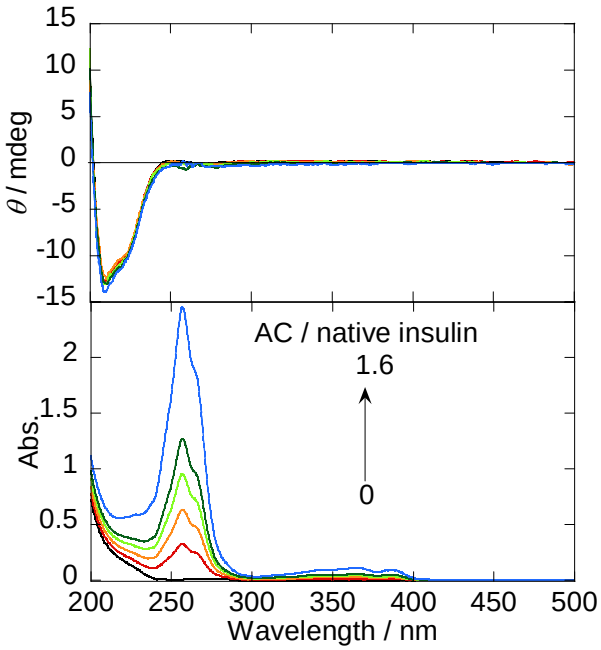

Fig. S2 UV-vis (bottom) and CD (top) spectral changes upon addition of 0-1.6 equivalents of AC to a phosphate buffer solution (pH 7.0, containing 0.1 M NaCl) of native insulin (12 μM) at 25 °C.

Photoreaction and product analysis. In a 5 mL volumetric flask, the stock solutions of IF and AC in PBS containing 0.1 M NaCl were mixed and filled up with PBS containing 0.1 M NaCl to make a sample solution at AC/IF = 5 ([IF] = 12 μM in insulin monomer, [AC] = 60 μM). The opaque solution was filtrated by using a membrane filter (Millipore, pore size: 0.45 μm) to leave IF deposit on the membrane, which was sandwiched by two quartz plates and then irradiated at room temperature for 60 min at wavelength >320 nm by using a 300 W high pressure mercury lamp equipped with a uranium sleeve, while the filtrate was subjected to UV-vis analysis for evaluating the amount of AC left in the bulk solution. The irradiated sample was soaked in PBS (100 mL), and the mixture was ultrasonicated for 10 min and left for ca. 12 h. A one mL aliquot of the resulting solution was added to an equal amount of acetonitrile. The mixture was stirred for 10 min at 60 °C and ultrafiltrated (Millipore Amicon Ultra NMWL, cut-off MW: 3 kDa) to give the first extract. The precipitate on the filter was subjected to the same treatment for two times to give the second and third extracts. Each, or combined, extract was subjected to chiral HPLC analysis on a tandem column of Cosmosil 5C_{18}-AR-II (Nacalai Tesque) and Chiralcel OJ-RH (Daicel) at 35 °C eluted with a 64:36 water-acetonitrile mixture containing 0.1 % TFA at a flow rate of 0.5 mL min^{-1}. The yields of cyclodimers **1**-**4** and the ee values of **2** and **3** were determined from the peak areas of the HPLC chromatogram obtained by using a fluorescence detector (excitation at 254 nm and monitoring at 420 nm).[3]

Independent HPLC analyses of the first to third extracts obtained by repeated extractions of the products from the irradiated AC sample bound to (-)-IF. In order to quantitatively examine the efficiency of extraction (recovery) from the photoirradiated AC-IF samples, the first to third extracts obtained above by

repeated extractions of the products from the enantiodifferentiating photocyclodimerization of AC mediated by (–)-IF were independently analyzed by chiral HPLC. As can be seen from Table S1, the recovery (the sum of remaining AC and cyclodimers **1**-**4**) obtained upon first to third extraction gradually decreased from 62% to 25% and then to 5%, amounting 92% in total, indicating a good material balance that most of the charged AC was recovered by the three repeated extractions. Practically all of the yields and ee's obtained are well within the experimental errors (±2% for yield and ±3% for ee); the only exception is the ee of **3** in the third extract, which however had only a limited impact on the overall ee, due to the small contribution in quantity of the third extract. It is also crucial that the most abundant first and second extracts gave essentially the same yields and ee's, determining the overall values. Hence, we decided to analyze only the combined, rather than each, extract in most photochemical runs.

Table S1. Independent HPLC analyses of the first to third extracts obtained by repeated extractions of the products from the irradiated AC sample bound to (-)-IF[a]

extraction run[b]	recovery/%[c]	relative yield/% (*ee*/%)[d]						HH/HT[e]
		1	**2**		**3**		**4**	
1	62	13	22	(*13*)	33	(*18*)	32	1.9
2	25	13	22	(*14*)	32	(*17*)	33	1.9
3	5	11	20	(*11*)	30	(*8*)	39	2.2
overall	92	13	22	(*13*)	32	(*17*)	33	1.9

[a] Irradiated at >320 nm for 60 min at room temperature on a membrane filter sandwiched by two quartz plates. [b] See Experimental. [c] Determined by using the calibration curves for **1** (representing all cyclodimers) and AC. [d] Determined by chiral HPLC; error in relative yield ±2%; error in ee ±3%. The positive sign for ee value, originally meaning the first elusion on chiral HPLC using an ODS + OJ-RH tandem column, now refers to the preferred formation of (*M*)-enantiomers of **2** and **3** (ref. 4). [e] HH/HT = (**3**+**4**)/(**1**+**2**).

References

(1) A. Loksztejn and W. Dzwolak, *J. Mol. Biol.*, 2008, **379**, 9.

(2) A. Loksztejn and W. Dzwolak, *J. Mol. Biol*., 2010, **395**, 643.

(3) M. Nishijima, T. Wada, K. Nagamori and Y. Inoue, *Chem. Lett.*, 2009, **38**, 726.

(4) A. Wakai, H. Fukasawa, C. Yang, T. Mori and Y. Inoue, *J. Am. Chem. Soc.*, 2012, **134**, 4990 & 10306 (erratum).