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Supporting information for article:

Structures of holo wild-type human cellular retinol-binding protein II (hCRBPII) bound to retinol and retinal

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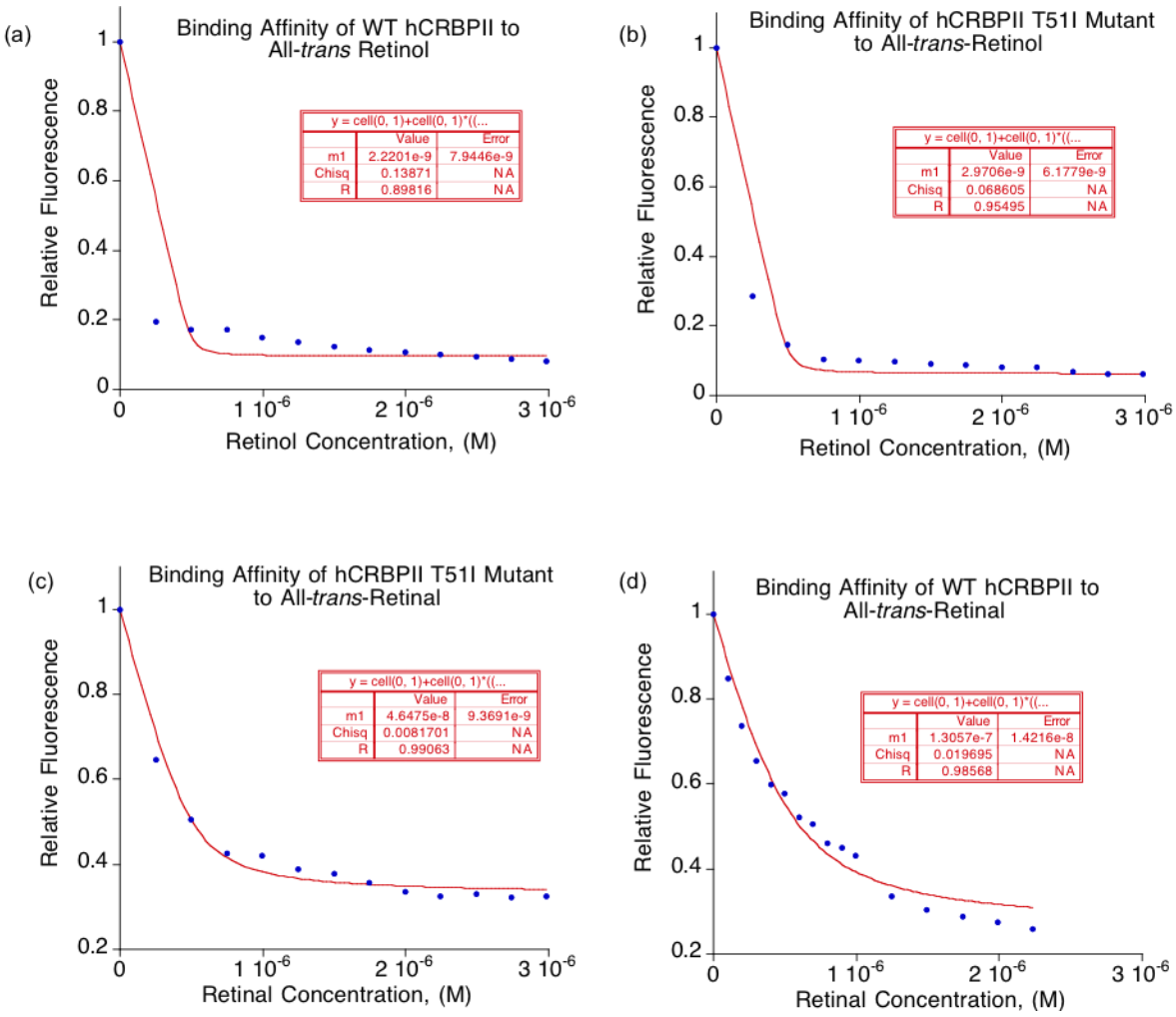

Figure S1 Measurement of ligand binding constants using the fluorescence quenching of tryptophan (excitation at 280 nm, emission at 350 nm). (a) Titration of WT-hCRBPII with all-*trans*-retinol. (b) Titration of hCRBPII T51I mutant with all-*trans*-retinol. (c) Titration of hCRBPII T51I mutant with all-*trans*-retinal. (d) Titration of WT-hCRBPII with all-*trans*-retinal.

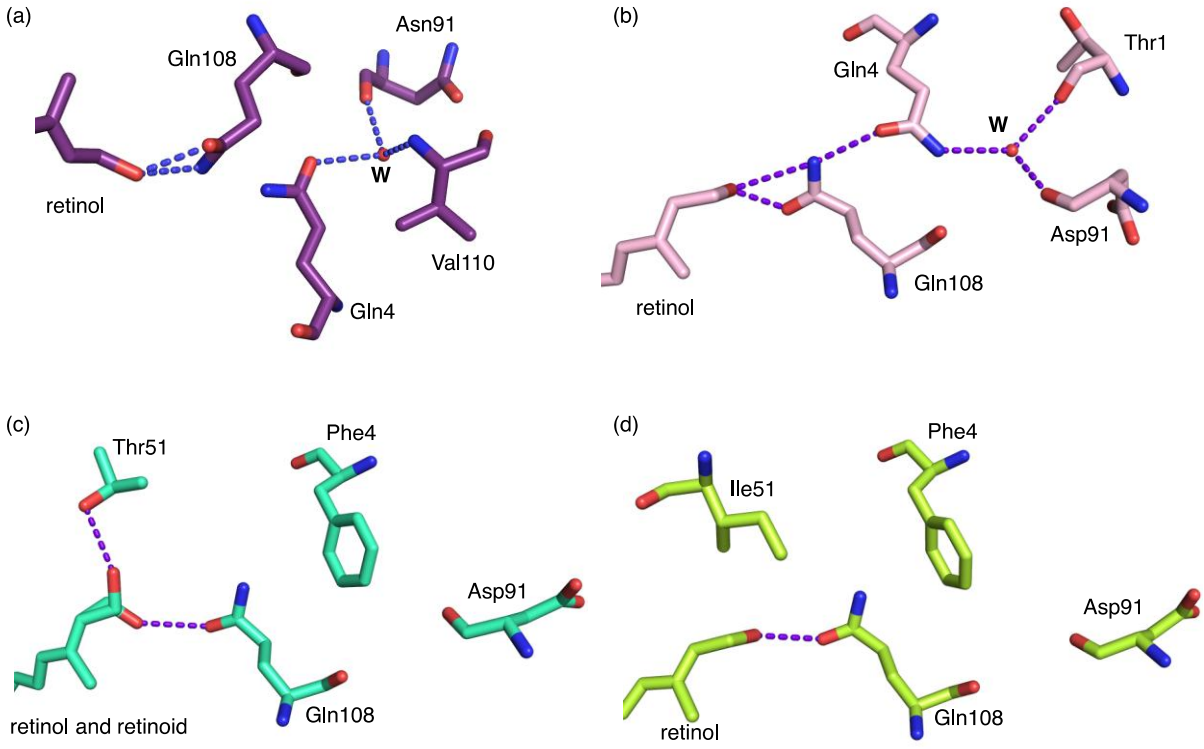

Figure S2 A comparison of interacting networks that include Gln108. (a) Crystal structure of retinol bound rat CRBPII (1OPB, purple). (b) Crystal structure of all-*trans*-retinol bound human CRBPII (4QZT, pink). (c) Crystal structure of zebra fish CRBP (1KQW, green). (d) Crystal structure of retinol bound rat CRBPI (1CRB, lime). Note the difference in the interactions to Gln108. Only in hCRBPII is the direction of the Gln108 sidechain defined by hydrogen bonding.

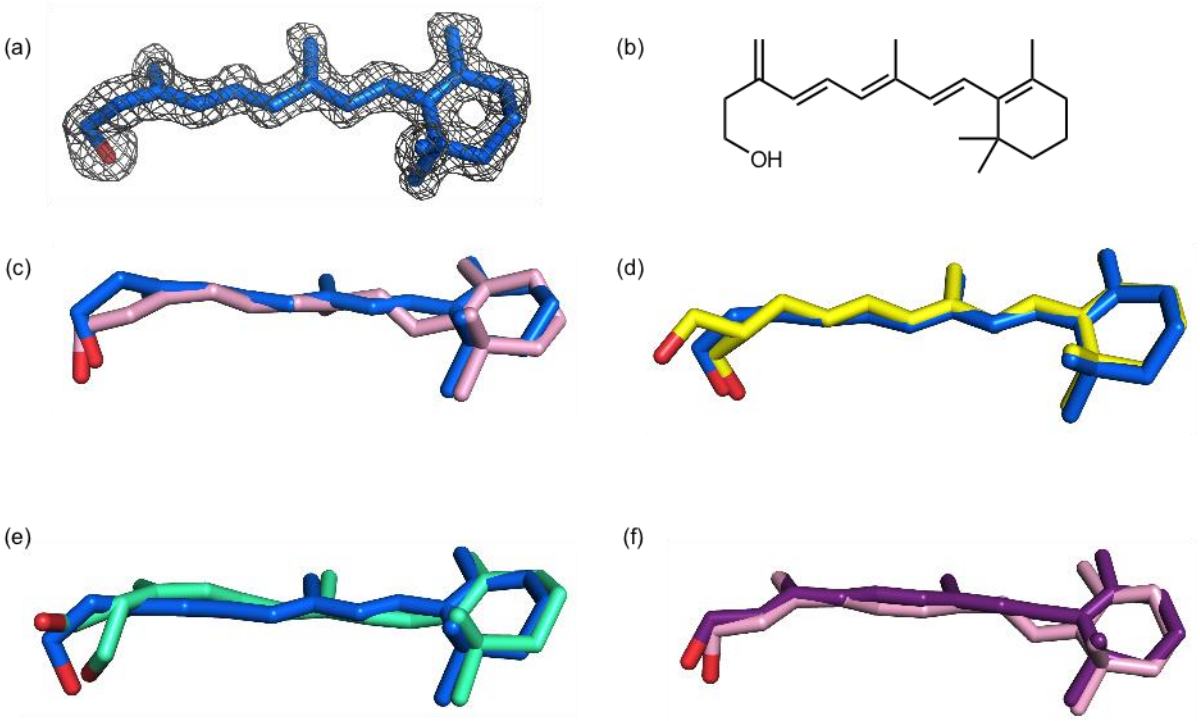

Figure S3 (a) The 2Fo-Fc electron density map contoured at 1.0 s around the rearranged retinol from data collected at 11 KeV and the highest flux (4QYN, blue). (b) The chemical structure of a possible retinoid derivative consistent with the crystallographic data. (c) The overlay of bonafide retinol structure (chain C) from data collected with an attenuated X-ray beam at an energy of 7 KeV (4QZT, Pink) and the structure of rearraged retinoid derivative (chain B) from data collected at 11 KeV and the highest flux (4QYN, blue). (d) The structures of the noncanonical retinoid (refined in two conformations) previously seen in the human CRBPII (2RCT, yellow), and our current rearranged structure (4QYN, blue) were overlaid. (e) The structures of the canonical and noncanonical retinoid (refined in two conformations) previously seen in the zebrafish CRBP (1KQW, green), and our current rearranged structure (4QYN, blue) were overlaid. (f) The overlaid structures of the canonical retinol previously obtained in the rat CRBPII (1OPB, purple) and our current bonafide all-*trans*-retinol structure in human CRBPII (4QZT, pink).

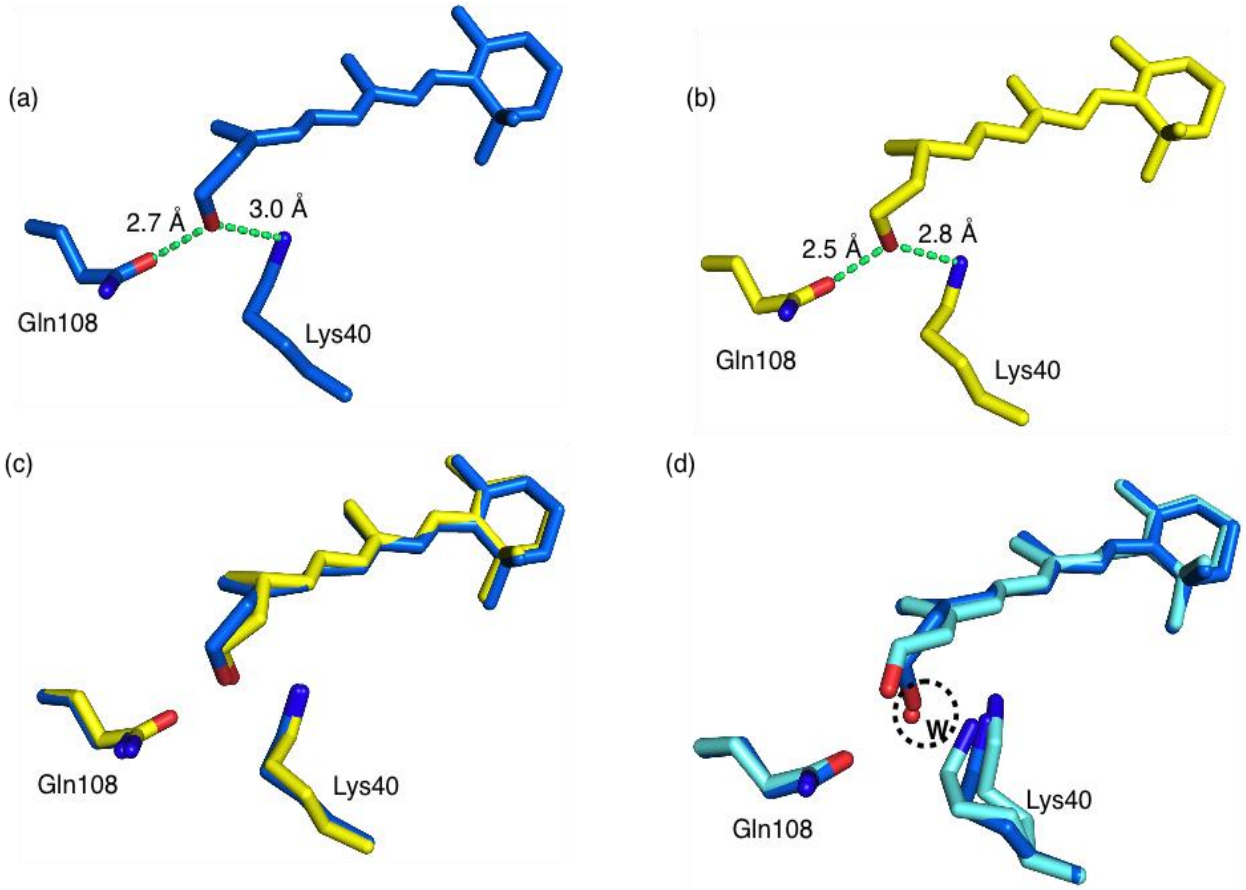

Figure S4 Crystallographic analyses of rearranged retinol structure. (a) The rearranged retinol from data collected at 11 KeV and the highest flux (4QYN, blue) and the interaction of alcohol moiety with the neighboring residues of Gln108 and Lys40. (b) The retinoid moiety found in the previously published hCRBPII (2RCT, yellow). The residues of Gln108 and Lys40 interact with the ligand similarly seen in (a). (c) The structures of the noncanonical retinoid previously seen in the human CRBPII (2RCT, yellow), and our current rearranged structure (4QYN, blue) were overlaid. (d) The structures of the retinal bound hCRBPII (4QYP, cyan, chain B), and our current rearranged retinol structure (4QYN, blue) were overlaid. The position of an ordered water molecule (**W**) seen in all-*trans*-retinal crystal structure is occupied by the hydroxyl group of the noncanonical retinoid (indicated in dashed circle). We observed same phenomenon in retinol structure from data collected at 7 KeV and an attenuated beam (see Figure 4d in the manuscript).

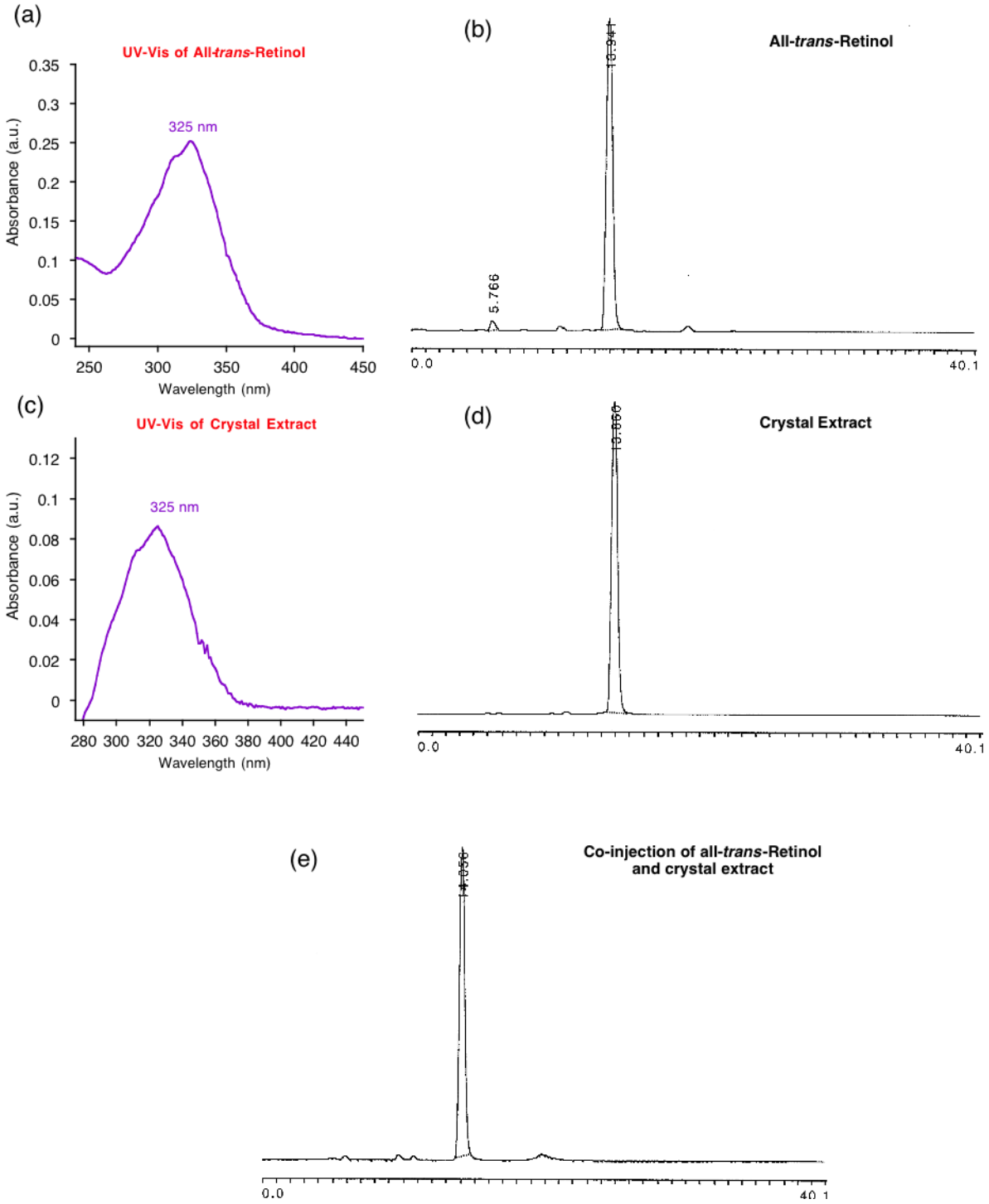

Figure S5 (a) UV-vis spectrum of all-*trans*-retinol. (b) The HPLC trace of all-*trans*-retinol. (c) UV-vis spectrum of the retinol extracted from the crystals and purified by HPLC. (d) HPLC chromatogram of the retinol extracted from crystals. (e) HPLC chromatogram of bonafide all-*trans*-retinol and the retinol extracted from crystals.

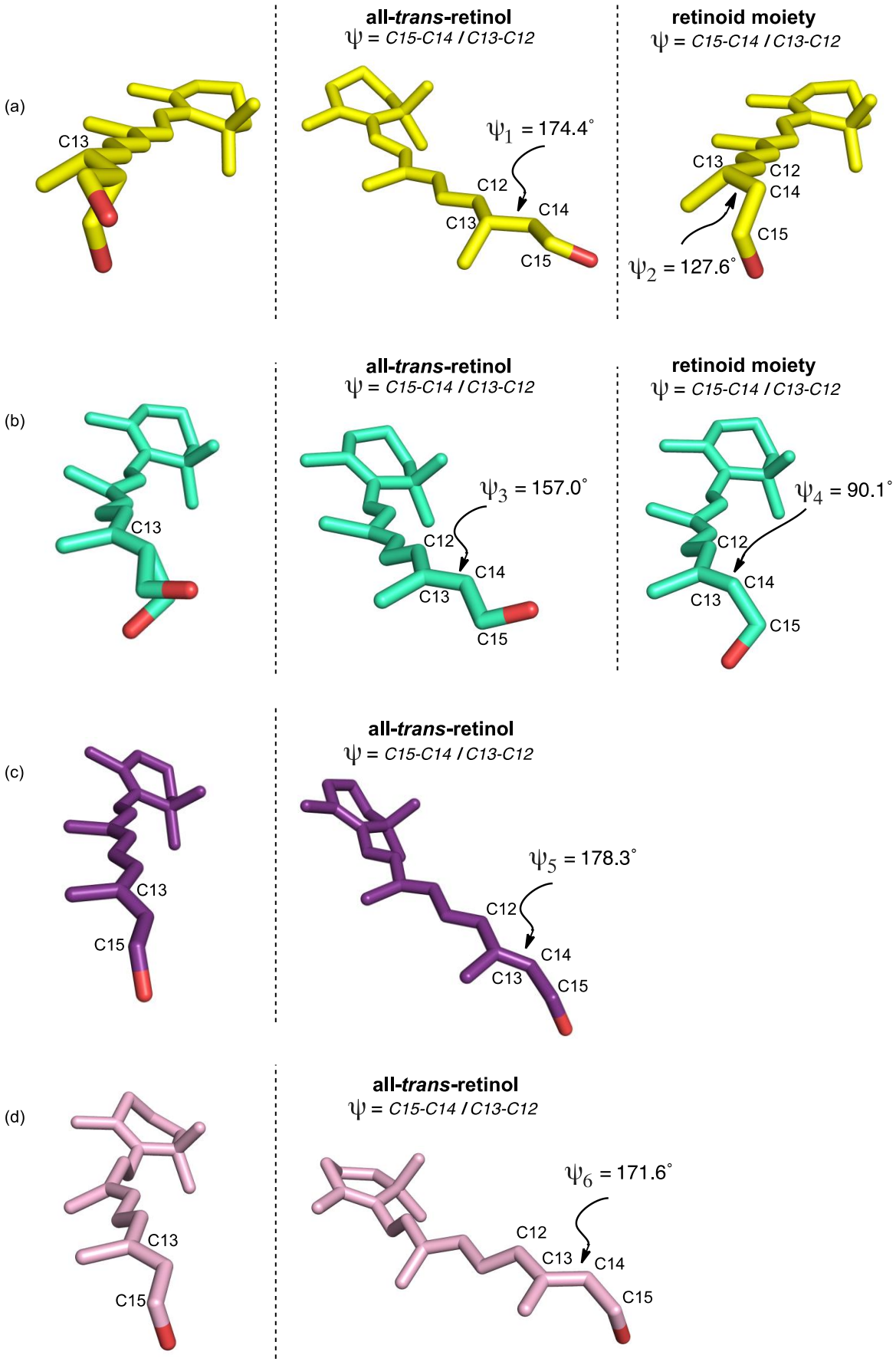
(a)
C13
all-*trans*-retinol
ψ = *C15-C14 / C13-C12*
ψ1 = 174.4°
C12
C14
C13
C15
retinoid moiety
ψ = *C15-C14 / C13-C12*
C13
C12
C14
C15
ψ2 = 127.6°
(b)
C13
all-*trans*-retinol
ψ = *C15-C14 / C13-C12*
ψ3 = 157.0°
C12
C14
C13
C15
retinoid moiety
ψ = *C15-C14 / C13-C12*
ψ4 = 90.1°
C12
C14
C13
C15
(c)
C13
C15
all-*trans*-retinol
ψ = *C15-C14 / C13-C12*
ψ5 = 178.3°
C12
C14
C13
C15
(d)
C13
C15
all-*trans*-retinol
ψ = *C15-C14 / C13-C12*
ψ6 = 171.6°
C12
C13
C14
C15

Figure S6 A comparison of the retinol and retinoid moieties found in the X-ray structures of human, zebra fish and rat CRBPII. (a) The retinol (left) and retinoid (right) moieties found in the previously published hCRBPII (2RCT, yellow). Note the 128° torsion angle (ψ) about the C13-C14 bond, a value inconsistent with a double bond. (b) The retinol (left) and retinoid (right) moieties seen in the crystal structure of zebrafish CRBP (1KQW, green). Again note the torsion angle about the C13-C14 bond, which is also inconsistent with a double bond. (c) The retinol structure in the rat CRBPII (1OPB, purple) (d) The retinol found in the X-ray structure of hCRBPII using data collected at an energy of 7 KeV and an attenuated beam (4QZT, pink).