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Supporting information for article:

Conformational flexibility within the small domain of human serine racemase

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Supplementary Table S1 Secondary structural elements in three human SR crystal structures.

The amino acids in element for the A-subunit in our holoenzyme structure were calculated by the PDBSUM server (see section 2.4). Note for 5X2L and 3L6B the 'amino acids in elements' are by default taken from the PDB header unless this disagrees with PDBSUM – in which case both definitions are given. The secondary structure names used in this paper are defined in the first column.

	amino acids in element			
Secondary structure element + sequence	A-subunit This pap./ PDBSUM	52XL PDBHead /PDBSUM	3L6B PDBHead /PDBSUM	COMMENT (sc = sidechain; mc = mainchain). [2.9 ± 0.1 Å — indicates three distances from three structures are all between 2.9 and 3.0 Å].
α1 SFADVEKAHINIR	8–20	8–20	8–20	Does not pack against central β-sheet of large domain. S8 is N-CAP.
H3_{10}–1 ISD	21–23	21–23	21–23	3_{10} helix – one 3_{10} H-bond C=O 19 to NH 22 (2.9 ± 0.1Å); C=O 19 to NH 23 is (3.3 ± 0.1 Å). S22 C=O accepts H-bond from K51 sc (K51 sc also donates to sc of Y93); sc of S22 interacts with R65 sc
β1 PVL	27–29	28–29	28–29	P27 carbonyl accepts H-bond from C48. L29 two H-bonds to F44.
α2 SSILNQLTG	31–39	31–39	31–39	
β2 NLFFKCE	41–47	41–46	41–46	Strand in middle of sheet. C46 NH donates H-bond to P27 (see below for C=O C46). NH E47 donates H-bond to both C=O L312 (and C=O K45). (Note 41–47 parallel to 306–312, but after 3_{10} helix and β-turn, then two antiparallel H-bonds from 53–315 and 55–313 – almost extend sheet; see below for details of H-bonds).
H3_{10}–2 LFQ	48–50	48–55	48–54 /48–50	NOTE. Although PDB header suggests one 3_{10} helix, this is not correct. PDBSUM gives two 3_{10} helices for all three structures. 48–50; C=O C46 accepts H-bond from F49 and C=O E47 accepts H-bond from Q50.
A type II β-turn (resides 50–53 QKTG) mis-assigned as	51–53	51–53	51–54	The mainchain NH G53 donates an H-bond to C=O of Q50 (C=O of G53 accepts an H-bond from mc NH of G315). All three

3_{10}-helix. An ERROR in PDB headers and PDBSUM. + 53–55				structures have similar phi-psi angles for residues in this type II turn: Q50, phi-psi (-70, 157), K51 (-53, 136), T52 (73, 172), G53 (92, 174). T52 is one of only three non-glycine residues with positive phi.
α3 KIRGALNAVRSL	56–67	56–66	55–66	F55 is in α-helical region of Ramachandran; note NH of F55 makes H-honds to O S313 and NH G515. The sc of K56 (to which PLP is covalently attached) has a slightly different conformer in malonate structure. In serine dehydratase helix is defined as being in small domain.
β3 AVVT (HS)	78–81	79–81 /78–81	78–82 /78–83	Apo holoenzyme structures β-strand is 78–81 (AVVT). In complex with malonate (3L6B) strand is 78–83 (AVVTHS). FIRST ELEMENT IN SMALL DOMAIN. **Loop 82–85 is different. H82 pep flips between malonate and apo.**
α4 GNHGQALTYAAKLEG	85–99	85–98	85–98 /86–98	G85 is in α-helical region of Ramachandran in apo structures. With Malonate it has a positive phi. It is different. **This small domain helix stays with large domain in superpositions.**
β4 PAYIVVPQ	101–108	102–107 /101–108	102–107 /101–108	β3 and β4 are central two strands of four parallel strands in small domain.
α5 PDCKKLAIQAY	111–121	111–121	111–121	This helix moves with small domain. Y121 at C-terminus is tucked in and packs against G88 and OH makes H-bond with C=O of S84 in apo structures. In malonate structure Y121 flips out and is in different position. In human serine dehydratase the sequence of this helix is completely different apart from N-terminal Pro.
β5 SIVYC	124–128	124–128	124–128	Edge-strand. H-bonds with 102–108 from β4.

α6 SDESRENVAKRVTEET	131–146	131–146	131–147	No major differences in conformation at C-terminal end of helix.
β6 IMV	149–151	149–151	149–151	Edge-strand. H-bonds with 78–80 from β3. Moves with small domain and superpose OK, but phi-psi all change in same direction. Strand bends. Apo I (-115, 140), M (-81, 141), V (-126, 103) cf Malonate I (-109, 124), M (-74, 126), V (-119, 94)
α7 EPAVIAGQGTIALEVLNQV	156–174	156–164/ 165–174	156–174	Helix α7 looks same in all three structures. One long helix 156–174.
β7 DALVVPV	178-184	179-183 /178-184	179-183 /178-184	End residues 178 and 184 each make one H-bond (to 205 and 209). Although P183 is in β-region of Ramachandran it makes no H-bonds.
α8 GGMLAGIAITVKALK	187–201	187–201	187–201	N-terminus of helix and proceeding two Glycine residues (GGGGM) all point NHs at phosphate from PLP.
β8 KVYAAEPS	205–212	205–211 /205–212	205–211 /205–212	S212 NH donates H-bond to V262 but is in α-region of Ramachandran. P211 is in β-region of Ramachandran but makes no H-bonds. Note sc E210, D216 and mc A214 coordinate metal.
α9 DDCYQSKLKG	215–224	215–224	215–224	Note D216 coordinates metal.
$H3_{10}$–3 ADGVK	237–241	237–241	237–241	240–237 H-bond OK. 241 NH to 238 H-bond? 3.77 Å.
α10 TWPIIRDLV	248–256	247–256	247–256	T248 is N-terminus of helix. N247 makes no mc H-bonds (though in α-region of Ramachandran)
β9 DIFTV	258–262	258–262	258–262	Edge-strand.
α11 TEDEIKCATQLVWERMK	263–279	263–279	263–279	Helix α11 is on outside, and packs over top of (burying part of) helix a12.
α12 EPTAGVGVAAVLS	283–295	286–295 /287–295	283–295	Some of helix is completely buried. 285–295 in α-helical region of Ramachandran – but some backbone H-bonds not perfect. PDB header for 5X2L has short 3_{10} helix from 283–285. Irregular H-bonds with some C=Os going to two NHs.

$H3_{10}$–4 QHFQTV	296–301	296–301	296–301	3_{10} helix. Main-chain NHs of QTV within H-bond distance of QHF respectively.
β10 KNICIVL	306–312	307–312 /306–312	307–312 /306–312	K306 accepts main-chain H-bond from NH of N41, (K306 in α-region of Ramachandran – all other residues in β-region).
$H3_{10}$–5 LTSSITW	319–325	**ABSENT**	320–324	C-terminal α13 helix is at the dimer interface. It moves by approx. 1.5 Å when apo and malonate structures are compared. **In human apo 5X2L structure C-terminal residue is V317 (in both subunits); this helix is absent.** High temperature factors suggest when this α13 helix is present it is often quite mobile. (The electron density maps for the C-terminal region of the rat *holo* 3HMK structure are not very clear. Note one indel before this helix rat cf human SR.

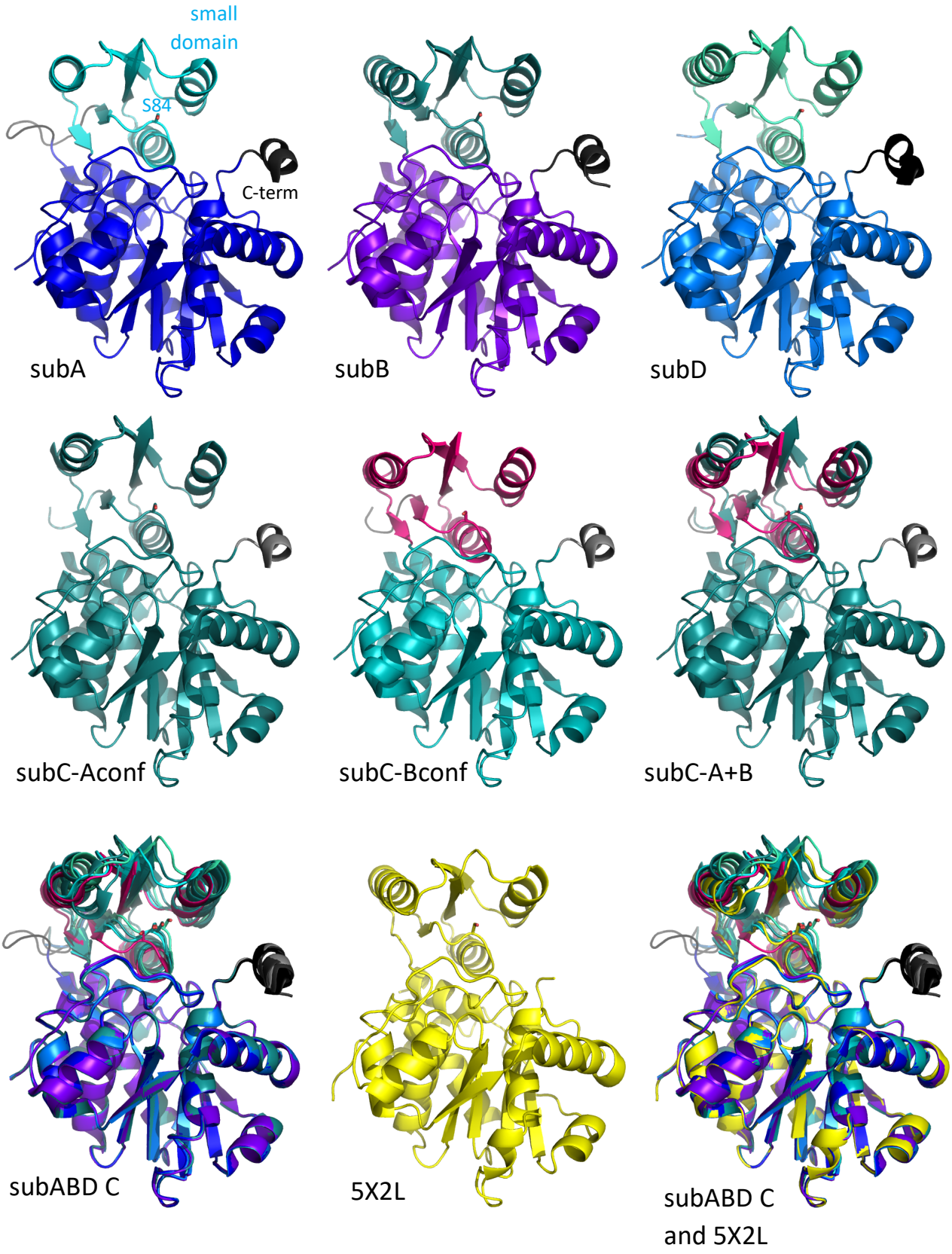
Supplementary Table S2. Phi-psi angles for the β3-α4 loop and for the β6 strand regions.

	Holoenzyme A-subunit		Malonate structure (3L6B)	
	phi	psi	phi	psi
THR 81A	*-157*	*162*	*-157*	*174*
HIS 82A	-92	-11	*-117*	*149*
SER 83A	-88	139	*-165*	*144*
SER 84A	-117	-7	-77	-9
GLY 85A	*-90*	*-133*	149	-44
ASN 86A	*-55*	*-39*	*-70*	*-47*
HIS 87A	*-69*	*-46*	*-64*	*-38*
GLY 148A	-88	175	-89	-177
ILE 149A	*-115*	*140*	*-109*	*124*
MET 150A	*-81*	*141*	*-74*	*126*
VAL 151A	*-126*	*103*	*-119*	*94*

Supplementary Table S3. Structural comparison of A subunit (*holo*) with malonate (3L6B) and *holo* (5X2L) hSR.

Regions compared (residue range)	Structure cf	R.M.S.D. Å	Nalign
All (4–316)	mal. (3L6B)	2.37	306
	holo (5X2L)	0.682	303
All β-strands	mal. (3L6B)	2.133	51
"	*holo* (5X2L)	0.415	51
SD (78–155)	**mal. (3L6B)**	**1.76**	**78**
"	***holo* (5X2L)**	**0.867**	**78**
SD (78–81, 101–155)	**mal. (3L6B)**	**0.64**	**54**
"	***holo* (5X2L)**	**0.869**	**54**
SD (78–81, 101–108, 124–128, 149–151)	**mal. (3L6B)**	**0.69**	**20**
"	***holo* (5X2L)**	**0.34**	**20**
LD (4–66, 156–316)	mal. (3L6B)	0.282	223
"	*holo* (5X2L)	0.180	224
LD (27–29, 41–47, 178–184, 205–212, 258–262, 306–312)	mal. (3L6B)	0.104	31
"	*holo* (5X2L)	0.090	31

SD = small domain, LD = large domain. All β-strands = (β1 residues 27–29, β2 41–47, **β3 78–81, β4 101–108, β5 124–128, β6 149–151,** β7 178–184, β8 205–212, β9 258–262, β10 306–312). Nalign = number of residues aligned.



Supplementary Figure S1. Conformational flexibility within the small domain of *holo* SR.
The structures of the four subunits in the asymmetric unit of 6SLH (new *holo* structure) are shown in cartoon view on first two lines. Note for the C-subunit the small domain was modelled in two positions. In the bottom left panel all four subunits are shown superposed on the large (lower) domain; the structure of an apo human structure (5X2L) is shown in the bottom middle panel; all structures are superposed on bottom right. Note that in the 5X2L structure the C-terminal helical region is missing (black in other structures).