The crystal structure of full-length Sizzled from *Xenopus laevis* yields insights into Wnt-antagonistic function of secreted frizzled-related proteins

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Table-S1 Data collection and refinement statistics for Sizzled crystals

	crystal for phasing	crystal for refinement
Data collection		
X-ray source	MicroMax-003	BL17U
Detector	Saturn 944	Quantum 315
Wavelength (Å)	1.54178	0.97915
Crystal-to-detector distance (mm)	70	300
Oscillation range (°)	360	180
Oscillation width per image (°)	0.3	1
Space group	$P4_32_12$	$P4_32_12$
Unit cell dimensions: a, b, c (Å)	70.218, 70.218, 132.404	70.131, 70.131, 132.584
Resolution range (Å)[§]	50-2.60 (2.64-2.60)	50-2.08 (2.12-2.08)
Unique reflections	10669	20594
Completeness (%)	98.6 (76.0)	99.3 (99.8)
$I/\sigma(I)$	77.9 (12.4)	30.1 (3.1)
Redundancy	25.6 (16.0)	6.0 (6.1)
R_{merge} (%)[a]	5.1 (24.7)	7.1 (95.6)
R_{meas} (%)[a]	5.2 (25.5)	7.7 (100.0)
R_{pim} (%)[a]	1.0 (6.1)	3.2 (42.3)
[a] $CC_{1/2}$		(0.824)
[a] CC^*		(0.950)
SAD phasing		
Resolution range (Å)	50-2.9	
$R_{anomalous}$ (%)	3.5	
Number of sulfur atoms	3	
Figure of merit	0.41	
Refinement		
Resolution range (Å)		19.86-2.08 (2.19-2.08)
R_{work}[b]		21.3 (27.7)
R_{free}[b]		23.0 (31.9)
Average *B*-factor for protein and solvent (Å^2)		52.0, 48.4
r.m.s.d. bond length (Å)		0.002
r.m.s.d. bond angle (°)		0.457
Ramachandran (%; favored, allowed, generally allowed, disallowed)		93.2, 6.8, 0, 0

[§]Values in parenthesis are for the highest resolution shell.

[a] $R_{merge} = \Sigma_{hkl}|I - \langle I \rangle|/\Sigma_{hkl}I$, where I is the intensity of unique reflection *hkl*, and $\langle I \rangle$ is the average over symmetry-related observations of unique reflection *hkl*. Please see the references (67-69) for the definitions of R_{pim}, R_{means}, $CC_{1/2}$ and CC^*.

[b] $R_{work} = \Sigma|F_o - F_c|/\Sigma F_o$, where F_o and F_c are the observed and calculated structure factors, respectively. R_{free} was calculated using 5% of the reflections set aside from refinement.

Table S2 Secondary structure prediction of human sFRPs

sFRPs	sequence	Predicted/determined
Sizzled	PYDTRTMIEQWLLINENCAQKLI	Helices α6 and α7
sFRP1	KKKDLKKLVLYLKNGADCPCHQL	helix
sFRP2	SERDLKKSVLWLKDSLQCTCEEM	helix
sFRP3	SLVNIPRDTVNLYTSSGCLCPPL	beta-strand
sFRP4	SSPIPRTQVPLITNSSCQCPHIL	beta-strand
sFRP5	KRKDTKRLVLHMKNGAGCPCPQLD	helix

Fig. S1. (A) Overview of the anomalous difference Fourier map (contoured at 3.0 σ) for heavy atoms in an asymmetric unit. One Sizzled molecule was observed in the asymmetric unit (CRD domain, cyan; NTR domain, salmon). Eight pairs of disulfide bonds are indicated using yellow sticks. Two sulfates are also represented using sticks. Purple spheres represent chlorides. (B) Close-up view of the squared area of (A). (C) Stereoview of the simulated annealing omit map (grey mesh) for the NTR domain (indicated in cartoons).

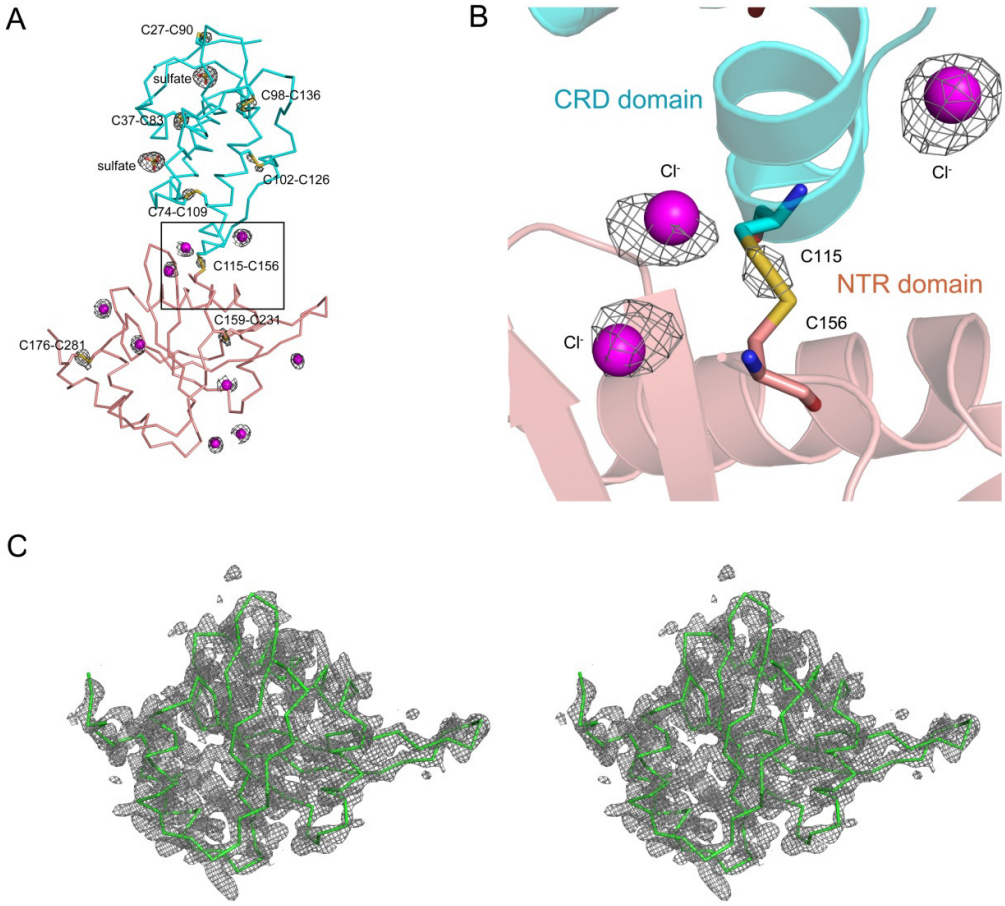

Fig. S1

Fig. S2. Sequence-based rooted phylogenetic analysis of *Xenopus laevis* Sizzled and human sFRPs. The sequence alignment was made by ClusterW and the phylogenetic tree was produced using TreeView.

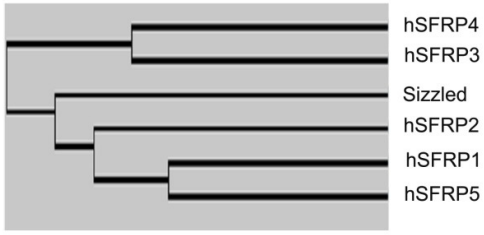

Fig. S3. (A) Side view of mouse sFRP3 CRD dimer (PDB code 1IJX) in ribbon model with two protomers in cyan and green, respectively. (B) Top view of the mouse sFRP3 CRD dimer with interfacial hydrophobic residues shown in stick representation.

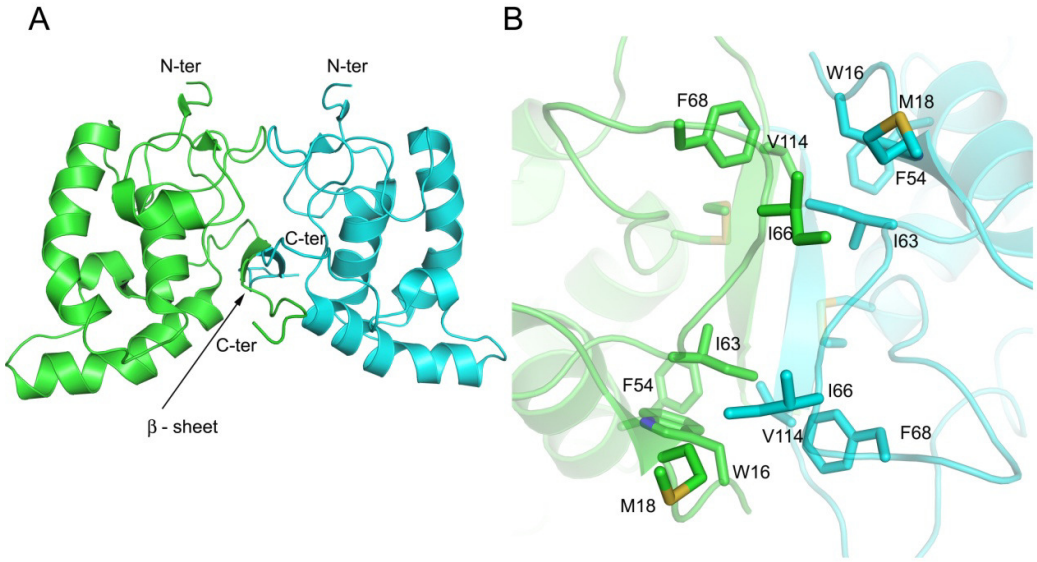

Fig. S3

Fig. S4. Supposed mechanism of biphasic modulation of Wnt signaling by sFRPs. (A) sFRPs (yellow) serve a carrier to carry free Wnt (green; with the PAM group in red) from a distant area to a target cell. The Wnt-sFRP complex diffuses to the local area to the target cell and binds to heparin or client proteins around the cells through the positively charged region (blue) on the bottom surface of the sFRP. The Wnt dissociated from the Wnt-sFRP complex binds to the CRD domain of Frizzled (purple) on the target cell for activating Wnt signaling. (B) sFRPs (yellow) diffuse to the local area of a target cell and serve a decoy to trap excess Wnt for inhibiting Wnt signaling. The diffusion of sFRPs depends on the existence of heparin or client proteins around the target cell.

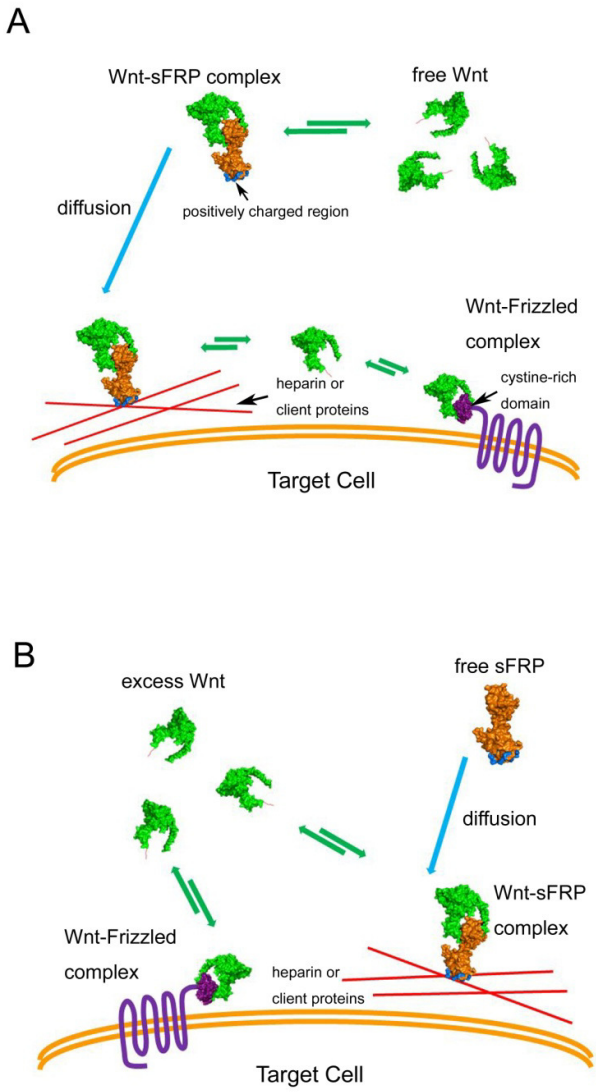
A
Wnt-sFRP complex
free Wnt
diffusion
positively charged region
Wnt-Frizzled
complex
heparin or
client proteins
cystine-rich
domain
Target Cell
B
excess Wnt
free sFRP
diffusion
Wnt-sFRP
complex
Wnt-Frizzled
complex
heparin or
client proteins
Target Cell

Fig. S4