

## Supplementary Information

### Structure of native lens connexin-46/50 intercellular channels by CryoEM

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## Supplementary Tables

### Supplementary Table 1

#### Summary of molecular dynamics simulation setup and conditions for Cx50, Cx46, Cx26 and Cx46/50 heteromeric/heterotypic models.

(\* n-terminal acetylation)

	Connexin-50	Connexin-50*	Connexin-46	Connexin-46*	Connexin-26	Connexin-26*
<b>Total Atoms</b>	364,952	363,722	360,190	355,524	368,756	367,346
Solvent	196,248	194,928	191,346	191,310	194,856	193,548
Lipids	133,866	133,866	133,732	129,042	133,464	133,330
Protein	34,440	34,488	34,716	34,764	39,972	40,020
Ions	398	440	396	408	464	448
Modelled Residues	2-97; 154-234	2-97; 154-234	2-97; 142-222	2-97; 142-222	1-109; 125-217	1-109; 125-217
<b>Simulation Conditions</b>						
Simulation Box (Å)	152 x 152 x 176	152 x 152 x 176	152 x 152 x 176	152 x 152 x 176	155 x 155 x 176	155 x 155 x 176
Pressure (atm)	1	1	1	1	1	1
Temperature (K)	310	310	310	310	310	310
Time Step (fs)	1	2	1	2	2	2
Equilibration Time (ns)	30	30	30	30	30	30
Production Time (ns)	100	70	70	50	50	70
seed # (10ns/seed)	2	6	2	5	3	4
Total Time (ns)	150	160	120	130	110	140

Connexin-46*/50* Models	Heterotypic	Heteromeric (I)	Heteromeric (II)
<b>Total Atoms</b>	363,884	361,412	361,412
Solvent	194,964	192,498	192,498
Lipids	133,866	133,866	133,866
Protein	34,626	34,626	34,626
Ions	428	422	422
Modelled Residues	Cx50: 2-97; 154-234 Cx46: 2-97; 142-222	2-97; 154-234 2-97; 142-222	2-97; 154-234 2-97; 142-222
Point Group Symmetry	C6	C3	D3
<b>Simulation Conditions</b>			
Simulation Box (Å)	152 x 152 x 176	152 x 152 x 176	152 x 152 x 176
Pressure (atm)	1	1	1
Temperature (K)	310	310	310
Time Step (fs)	2	2	2
Equilibration Time (ns)	30	30	30
Production Time (ns)	80	70	70
seed #	16	4	4
Total Time (ns)	270	140	140

Asterisk indicates models that included modification by n-terminal acetylation. Connexin-26 models were derived from PDB 2ZW3<sup>10</sup>.

## Supplementary Table 2

Local resolution assessment of a calculated map based on the Cx46 atomic model versus the 3.4 Å resolution CryoEM density map.

NTH														
G2	D3	W4	S5	F6	L7	G8	R9	L10	L11	E12	N13	A14	Q15	E16
3.0-4.4	3.0-4.6	2.8-3.2	2.9-3.3	3.4-6.7	2.9-3.0	2.9-3.9	3.2-4.2	3.2-3.3	2.8-3.2	3.1-5.4	3.4-5.2	3.1-3.5	2.8-3.2	3.6-6.4
TM1														
H17	S18	T19	V20	I21	G22	K23	V24	W25	L26	T27	V28	L29	F30	I31
3.0-3.3	2.9-3.0	2.8-3.2	2.8-3.0	3.1-3.2	2.8-2.9	2.8-3.1	3.0-3.1	2.8-3.1	2.8-3.0	3.0-3.4	2.8-2.9	2.8-2.9	2.8-3.3	2.9-3.4
TM1														
F32	R33	I34	L35	V36	L37	G38	A39	A40	A41	E42	E43	V44	W45	G46
2.8-3.0	2.7-3.0	2.8-3.0	2.8-3.0	2.8-3.0	2.9-3.0	2.9-3.0	2.8-2.9	2.9-3.2	2.8-2.9	3.0-9.0	2.8-3.7	2.8-2.9	2.6-2.8	2.8-3.0
EC1							EC1 Helix							
D47	E48	Q49	S50	D51	F52	T53	C54	N55	T56	Q57	Q58	P59	G60	C61
2.9-3.1	2.8-3.0	2.8-3.0	3.0-3.3	2.9-3.2	2.7-3.0	2.9-3.4	3.0-3.2	3.0-3.1	3.1-3.4	2.9-3.2	3.0-3.5	3.3-3.6	3.0-3.4	3.1-3.5
EC1 Helix							TM2							
E62	N63	V64	C65	Y66	D67	R68	A69	F70	P71	I72	S73	H74	V75	R76
3.0-6.6	3.0-3.1	3.0-3.1	2.9-3.2	2.7-3.0	3.2-3.7	3.0-7.2	2.9-3.0	2.7-3.2	2.8-3.1	2.9-3.1	2.9-3.1	3.0-3.1	2.8-2.9	2.9-3.0
TM2														
F77	W78	V79	L80	Q81	I82	I83	F84	V85	S86	T87	P88	T89	L90	I91
2.8-3.1	2.8-3.0	2.7-2.9	2.8-2.9	2.8-3.1	2.8-3.0	2.7-2.8	2.8-3.0	2.7-3.0	2.8-2.9	3.0-3.2	2.8-3.3	2.8-3.1	3.1-3.3	3.1-4.1
TM2						ICL	TM3							
Y92	L93	G94	H95	V96	L97	L142	L143	R144	T145	Y146	V147	F148	N149	
3.0-3.3	3.0-3.1	3.1-4.0	4.5-7.9	3.1-3.4	3.0-3.2	3.3-3.8	3.3-3.6	3.6-6.1	3.3-3.7	2.8-3.3	3.2-3.8	3.2-7.0	2.9-3.0	
TM3														
I150	I151	F152	K153	T154	L155	F156	E157	V158	G159	F160	I161	A162	G163	Q164
2.9-3.2	3.2-3.3	3.0-5.8	2.7-3.0	3.1-3.2	3.2-3.7	3.0-3.7	3.0-3.2	3.2-3.6	4.1-4.4	2.9-3.3	3.3-3.7	4.0-4.4	3.4-4.2	2.9-3.2
TM3							EC2							
Y165	F166	L167	Y168	G169	F170	Q171	L172	K173	P174	L175	Y176	R177	C178	D179
2.9-5.3	3.1-6.5	2.9-3.0	2.7-3.2	2.9-3.0	2.9-7.2	2.9-3.7	2.8-2.9	2.9-3.5	2.8-2.9	3.0-3.2	2.8-3.2	2.8-2.9	2.9-3.1	3.1-4.0
EC2							EC2							
R180	W181	P182	C183	P184	N185	T186	V187	D188	C189	F190	I191	S192	R193	P194
3.0-3.7	2.9-3.8	3.1-3.9	3.0-3.3	3.0-3.2	2.8-3.4	3.2-3.6	3.2-3.3	3.0-3.2	2.9-3.2	2.7-3.1	2.7-3.2	3.0-3.2	2.9-3.1	2.8-3.1
TM4														
T195	E196	K197	T198	I199	F200	I201	L202	F203	M204	L205	A206	V207	A208	C209
2.9-3.2	2.9-3.0	2.8-3.0	3.1-3.4	2.9-3.0	2.7-2.9	2.9-3.1	2.8-3.0	2.9-3.1	2.8-3.0	3.0-3.2	3.0-3.2	2.7-2.9	2.9-3.0	2.9-7.2
TM4													CTD	
V210	S211	L212	L213	L214	N215	V216	L217	E218	I219	Y220	H221	L222		
2.9-3.0	2.9-3.0	2.9-3.0	3.0-3.5	2.9-3.0	2.8-3.0	2.9-3.1	3.0-3.1	2.9-3.1	3.2-3.3	3.2-6.6	3.3-3.4	3.1-4.4		

Values indicated by each residue correspond to the range of resolutions (Å) reported for amino acid sidechain densities using BlocRes<sup>70</sup>. Secondary structure and domain labels are indicated for the n-terminal helix (NTH), transmembrane helices (TM1-4) and extracellular domains (EC1-2) intracellular loop (ICL) and C-terminal domain (CTD).

### Supplementary Table 3

Local resolution assessment of a calculated map based on the Cx50 atomic model versus the 3.4 Å resolution CryoEM density map.

NTH														
G2	D3	W4	S5	F6	L7	G8	N9	I10	L11	E12	E13	V14	N15	E16
3.8-4.2	3.0-5.7	2.8-3.0	3.0-3.2	3.4-5.8	2.8-3.0	3.2-3.8	3.1-4.3	3.3-3.6	2.9-3.0	3.2-4.9	3.4-6.0	3.2-3.7	3.2-4.7	3.4-5.4
TM1														
H17	S18	T19	V20	I21	G22	R23	V24	W25	L26	T27	V28	L29	F30	I31
3.2-4.3	3.0-3.1	3.3-3.9	3.2-3.7	3.3-3.4	3.6-4.0	3.2-6.6	3.0-3.1	3.0-3.4	3.0-3.8	3.1-3.3	2.8-3.1	2.9-3.1	2.9-3.6	3.0-3.3
TM1														
F32	R33	I34	L35	I36	L37	G38	T39	A40	A41	E42	F43	V44	W45	G46
2.9-3.2	2.9-3.0	2.9-3.1	2.9-3.0	2.9-3.0	2.9-3.0	2.8-3.0	2.8-3.7	3.0-3.2	2.8-2.9	3.0-5.0	3.8-5.7	2.7-2.8	2.6-2.8	2.7-2.9
EC1							EC1 Helix							
D47	E48	Q49	S50	D51	F52	V53	C54	N55	T56	Q57	Q58	P59	G60	C61
3.0-3.2	2.7-2.9	2.8-3.0	3.0-3.4	3.0-4.3	2.8-3.0	3.0-3.4	3.0-3.2	3.0-3.2	2.9-3.2	2.9-3.1	3.0-3.4	3.2-3.5	3.0-3.3	3.1-3.4
EC1 Helix							TM2							
E62	N63	V64	C65	Y66	D67	E68	A69	F70	P71	I72	S73	H74	I75	R76
3.1-8.0	3.1-3.2	2.9-3.2	3.8-3.1	2.8-3.0	3.0-3.2	2.7-3.4	2.0-3.5	2.7-3.2	2.8-2.9	2.8-3.1	2.7-3.0	3.0-3.1	2.8-3.0	3.9-3.2
TM2														
L77	W78	V79	L80	Q81	I82	I83	F84	V85	S86	T87	P88	S89	L90	V91
2.8-2.9	2.7-2.9	2.7-2.9	2.8-3.0	2.8-3.1	2.9-3.0	2.8-3.0	2.8-2.9	2.8-3.0	2.8-3.1	3.0-3.2	2.8-3.3	2.7-3.2	3.1-3.3	3.0-3.1
TM2						ICL		TM3						
Y92	L93	G94	H95	A96	V97	L154	L155	R156	T157	Y158	V159	C160	H161	
3.2-3.6	3.0-3.1	3.1-4.1	3.2-8.0	3.3-3.5	3.1-4.3	3.2-3.5	3.2-3.4	3.5-7.0	4.0-4.9	2.9-3.5	3.2-4.0	3.1-4.2	2.8-3.8	
TM3														
I162	I163	F164	K165	T166	L167	F168	E169	V170	G171	F172	I173	V174	G175	H176
3.3-4.0	3.2-4.0	3.0-3.6	2.8-3.6	3.0-3.2	3.3-4.2	2.8-3.7	3.1-6.0	3.1-3.3	4.2-4.6	2.9-4.5	3.2-4.5	3.1-4.3	4.0-4.7	4.0-6.5
TM3							EC2							
Y177	F178	L179	Y180	G181	F182	Q183	I184	L185	P186	L187	Y188	R189	C190	S191
3.0-6.5	3.8-6.3	3.0-3.9	2.7-3.5	2.8-2.9	2.8-7.2	2.8-7.2	2.9-3.1	2.9-3.6	2.9-3.0	2.9-3.2	2.8-3.0	2.8-3.4	2.8-3.1	3.0-3.1
EC2														
R192	W193	P194	C195	P196	N197	V198	V199	D200	C201	F202	V203	S204	R205	P206
3.0-5.7	3.0-4.0	2.9-3.9	3.0-3.2	3.0-3.2	3.2-3.7	3.1-3.7	3.2-3.4	3.0-3.2	3.0-3.1	2.8-2.9	2.8-3.1	3.1-3.2	2.8-2.9	2.9-3.1
TM4														
T207	E208	K209	T210	I211	F212	I213	L214	F215	M216	L217	S218	V219	A220	S221
3.9-3.1	2.9-3.3	2.8-2.9	2.9-3.1	2.9-3.0	2.8-2.9	3.0-3.1	2.8-2.9	3.0-3.1	2.9-3.0	2.9-3.2	3.1-3.2	2.9-3.0	2.9-3.1	2.9-3.3
TM4												CTD		
V222	S223	L224	F225	L226	N227	I228	L229	E230	M231	S232	H233	L234		
3.0-3.1	2.9-3.0	3.0-3.1	2.7-7.2	2.9-3.0	2.9-3.1	3.0-5.3	3.1-6.0	3.1-3.3	3.1-6.5	3.5-4.4	3.3-3.6	3.3-4.7		

Values indicate by each residue correspond to the range of resolutions (Å) reported for amino acid sidechain densities using BlocRes<sup>70</sup>. Secondary structure and domain labels are indicated for the n-terminal helix (NTH), transmembrane helices (TM1-4) and extracellular domains (EC1-2) intracellular loop (ICL) and C-terminal domain (CTD).