Supplementary Information

Cryptochrome-Timeless structure reveals circadian clock timing mechanisms

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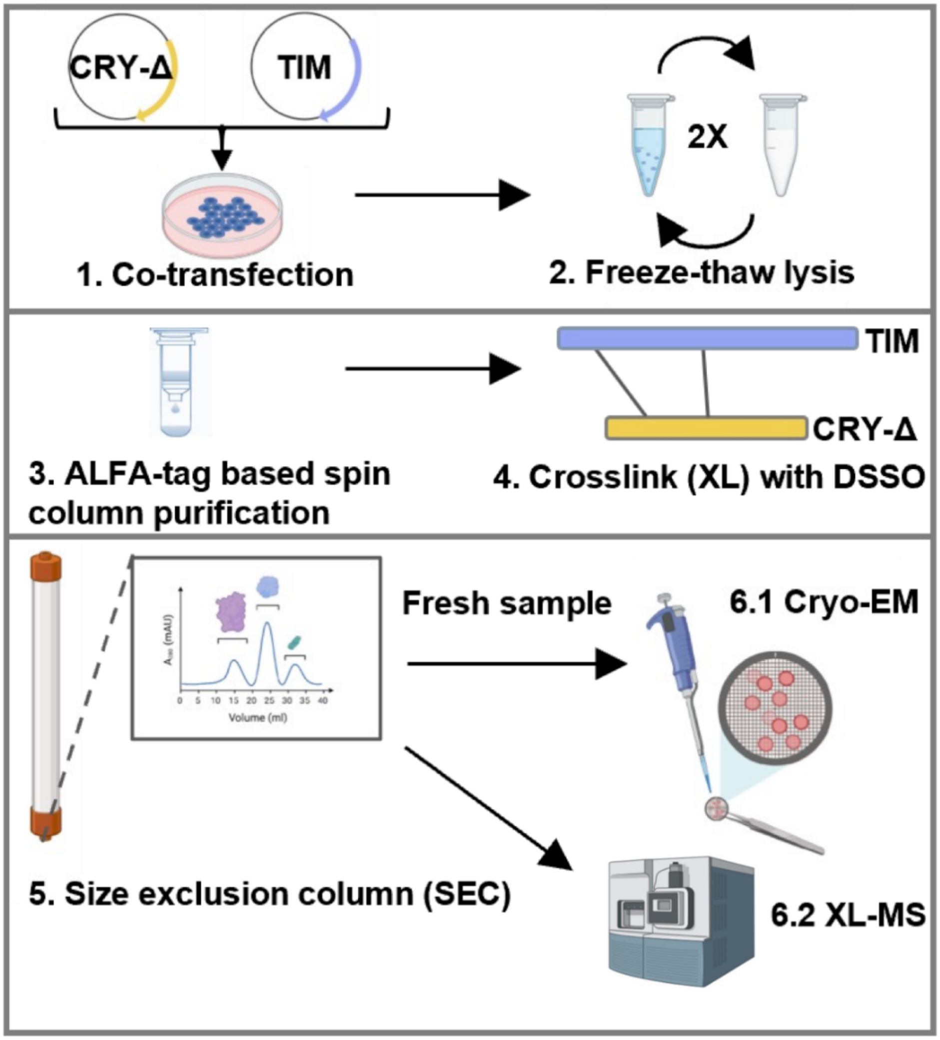

Methods Overview

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DNA constructs:

TIM-SNAP-HA in pAC5.1 vector backbone (with unintentional minor differences from standard pAC5.1)

Key features:
Ampicillin Resistance: 171-1136
Actin 5C promoter: 1286-3794
Drosophila Kozak sequence: 3805-3812
TIM: 3809-8002
SNAP-tag: 8021-8566
HA-tag: 8576-8602

ALFA-tag: 8624-8662
Replication origin: 9471-10059

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CLIP-CRYΔ in pAC5.1 vector backbone (with unintentional minor differences from standard pAC5.1)

Key features:
Ampicillin Resistance: 171-1136
Actin 5C promoter: 1286-3794
Drosophila Kozak sequence: 3807-3814
3X myc-tag: 3814-3921

CLIP-tag: 3931-4476
CRYΔ: 4489-6048
Replication origin: 6944-7532

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GGCATTGGCCAAGCGACTTGATCCGGATGGCACCTACATCAAGCAGTACGTCCCGGAGTTGATGAATGTG
CCCAAGGAATTTGTTCACGAGCCCTGGCGAATGTCTGCCGAGCAGCAGGAGCAGTACGAGTGCCTGATCG
GAGTCCATTATCCGGAGCGGATCATTGATTTGTCCATGGCCGTAAAGCGAAACATGCTGGCCATGAAGTC
TCTCCGAAATTCGCTGATCACCCCGCCGTAATCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCT
CTCCTCGGTCTCGATTCTACGCGTACCGGTCATCATCACCATCACCATTGAGTTTAAACCCGCTGATCAG
CCTCGACTGTGCCTTCTAAGGCCTGAGCTAGCTGATCAGCCTCGATCGAGGATCCAGACATGATAAGATA
CATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGAT
GCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTA
TGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGC
TGATTATGATCAGTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTCAGCTCCTTCCGGTGGGCGCGGGG
CATGACTATCGTCGCCGCACTTATGACTGTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGGCAGCG
CTCTGGGTCATTTTCGGCGAGGACCGCTTTCGCTGGAGCGACGATGATCGGCCTGTCGCTTGCGGTATTC
GGAATCTTGCACGCCCTCGCTCAAGCCTTCGTCACTGGTCCCGCCACCAAACGTTTCGGCGAGAAGCAGG
CCATTATCGCCGGCATGGCGGCCGACGCGCTGGGCTACGTCTTGCTGGCGTTCGCGACGCGAGGCTGGAT
GGCCTTCCCCATTATGATTCTTCTCGCTTCCGGCGGCATCGGGATGCCCGCGTTGCAGGCCATGCTGTCC
AGGCAGGTAGATGACGACCATCAGGGACAGCTTCAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCG
CGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAG
GTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCT
GTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATA

GCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCC
CGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTA
TCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCT
TGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGT
TACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTT
GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAA

Proteins Sequences:

CLIP-CRYΔ (MW = 83.7 kDa)

MEQKLISEEDLGGGEQKLISEEDLGGGEQKLISEEDLGGGMDKDCEMKRTTLDSPLGKLELSGC
EQGLHRIIFLGKGTSAADAVEVPAPAAVLGGPEPLIQATAWLNAYFHQPEAIEEFPVPALHHPV
FQQESFTRQVLWKLLKVVKFGEVISESHLAALVGNPAATAAVNTALDGNPVPILIPCHRVVQGD
SDVGPYLGGLAVKEWLLAHEGHRLGKPGLGGGSGMATRGANVIWFRHGLRLHDNPALLAALADK
DQGIALIPVFIFDGESAGTKNVGYNRMRFLLDSLQDIDDQLQAATDGRGRLLVFEGEPAYIFRR
LHEQVRLHRICIEQDCEPIWNERDESIRSLCRELNIDFVEKVSHTLWDPQLVIETNGGIPPLTY
QMFLHTVQIIGLPPRPTADARLEDATFVELDPEFCRSLKLFEQLPTPEHFNVYGDNMGFLAKIN
WRGGETQALLLLDERLKVEQHAFERGFYLPNQALPNIHDSPKSMSAHLRFGCLSVRRFYWSVHD
LFKNVQLRACVRGVQMTGGAHITGQLIWREYFYTMSVNNPNYDRMEGNDICLSIPWAKPNENLL
QSWRLGQTGFPLIDGAMRQLLAEGWLHHTLRNTVATFLTRGGLWQSWEHGLQHFLKYLLDADWS
VCAGNWMWVSSSAFERLLDSSLVTCPVALAKRLDPDGTYIKQYVPELMNVPKEFVHEPWRMSAE
QQEQYECLIGVHYPERIIDLSMAVKRNMLAMKSLRNSLITPP

TIM-SNAP-HA-ALFA (MW = 179.8 kDa)

MDWLLATPQLYSAFSSLGCLEGDTYVVNPNALAILEEINYKLTYEDQTLRTFRRAIGFGQNVRS
DLIPLLENAKDDAVLESVIRILVNLTVPVECLFSVDVMYRTDVGRHTIFELNKLLYTSKEAFTE
ARSTKSVVEYMKHILESDPKLSPHKCDQINNCLLLLRNILHIPETHAHCVMPMMQSMPHGISMQ
NTILWNLFIQSIDKLLLYLMTCPQRAFWGVTMVQLIALIYKDQHVSTLQKLLSLWFEASLSESS
EDNESNTSPPKQGSGDSSPMLTSDPTSDSSDNGSNGRGMGGGMREGTAATLQEVSRKGQEYQNA
MARVPADKPDGSEEASDMTGNDSEQPGSPEQSQPAGESMDDGDYEDQRHRQLNEHGEEDEDEDE
VEEEEYLQLGPASEPLNLTQQPADKVNNTTNPTSSAPQGCLGNEPFKPPPPLPVRASTSAHAQM
QKFNESSYASHVSAVKLGQKSPHAGQLQLTKGKCCPQKRECPSSQSELSDCGYGTQVENQESIS
TSSNDDDGPQGKPQHQKPPCNTKPRNKPRTIMSPMDKKELRRKKLVKRSKSSLINMKGLVQHTP
TDDDISNLLKEFTVDFLLKGYSYLVEELHMQLLSNAKVPIDTSHFFWLVTYFLKFAAQLELDME
HIDTILTYDVLSYLTYEGVSLCEQLELNARQEGSDLKPYLRRMHLVVTAIREFLQAIDTYNKVT
HLNEDDKAHLRQLQLQISEMSDLRCLFVLLLRRFNPSIHSKQYLQDLVVTNHILLLILDSSAKL
GGCQTIRLSEHITQFATLEVMHYYGILLEDFNNNGEFVNDCIFTMMHHIGGDLGQIGVLFQPII
LKTYSRIWEADYELCDDWSDLIEYVIHKFMNTPPKSPLTIPTTSLTEMTKEHNQEHTVCSWSQE
EMDTLYWYYVQSKKNNDIVGKIVKLFSNNGNKLKTRISIIQQLLQQDIITLLEYDDLMKFEDAE
YQRTLLTTPTSATTESGIEIKECAYGKPSDDVQILLDLIIKENKAQHLLWLQRILIECCFVKLT
LRSGLKVPEGDHIMEPVAYHCICKQKSIPVVQWNNEQSTTMLYQPFVLLLHKLGIQLPADAGSI
FARIPDYWTPETMYGLAKKLGPLDKLNLKFDASELEDATASSPSRYHHTGPRNSLSSVSSLDVD
LGDTEELALIPEVDAAVEKAHAMASTPSPSEIFAVPKTKHCNSIIRYTPDPTPPVPNWLQLVMR
SKCNHRTGPSGDPSDCIGSSSTTVDDEGFGKSISAATSQAASTSMSTVNPTTTLSLNMLNTFMG
SHNENSSSSGCGGTVSSLSMVALMSTGAAGGGGNTSGLEMDVDASMKSSFERLEVNGSHFSRAN

NLDQEYSAMVASVYEKEKELNSDNVSLASDLTRMYVSDEDDRLERTEIRVPHYHLEGGSGMDKD
CEMKRTTLDSPLGKLELSGCEQGLHRIIFLGKGTSAADAVEVPAPAAVLGGPEPLMQATAWLNA
YFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKT
ALSGNPVPILIPCHRVVQGDLDVGGYEGGLAVKEWLLAHEGHRLGKPGLGGGGYPYDVPDYART
GGGSGSRLEEELRRRLTE

Supplementary Table 1. Cryo-EM data collection, refinement, and validation statistics

	CRY:TIM complex (EMDB-27335) (PDB 8DD7)
Data collection and processing	
Magnification	x79k
Voltage (kV)	200
Electron exposure (e–/Å^2)	53
Defocus range (μm)	-0.8 ~ -2.0
Pixel size (Å)	1.032
Symmetry imposed	C1
Initial particle images (no.)	334,787
Final particle images (no.)	159,544
Map resolution (Å)	3.3
FSC threshold	0.143
Map resolution range (Å)	2.4-5.5
Refinement	
Initial model used (PDB code)	4GU5
Model resolution (Å)	3.3
FSC threshold	0.143
Model resolution range (Å)	2.4-5.5
Map sharpening *B* factor (Å^2)	-122.7
Model composition	
Non-hydrogen atoms	10370
Protein residues	1275
Ligands	FAD: 1
B factors (Å^2)	
Protein	132
Ligand	58
R.m.s. deviations	
Bond lengths (Å)	0.006 (6)
Bond angles (°)	1.185 (10)
Validation	
MolProbity score	2.00
Clashscore	11.07
Poor rotamers (%)	0.44
Ramachandran plot	
Favored (%)	93.27
Allowed (%)	6.73
Disallowed (%)	0.00