

Arginine tuning of coiled-coil peptides yields single-domain nanocrystals

Tanja Weil

`tweil@mpip-mainz.mpg.de`

Max Planck Institute for Polymer Research <https://orcid.org/0000-0002-5906-7205>

Patrick Roth

Max Planck Institute for Polymer Research

Iain Harley

University of Glasgow <https://orcid.org/0000-0002-3767-4450>

Marco Santucci

Johannes Gutenberg University Mainz <https://orcid.org/0000-0002-7367-4343>

Katherine Albanese

University of Bristol

Zahra Riazimand

Max Planck Institute for Polymer Research

Ingo Lieberwirth

Max-Planck Institute for Polymer Research <https://orcid.org/0000-0003-1323-524X>

Ute Kolb

Johannes Gutenberg University Mainz <https://orcid.org/0000-0003-0559-2482>

David Ng

Max Planck Institute for Polymer Research <https://orcid.org/0000-0002-0302-0678>

Katharina Landfester

Max Planck Institute for Polymer Research <https://orcid.org/0000-0001-9591-4638>

Thomas Sharp

University of Bristol <https://orcid.org/0000-0002-1990-2333>

Derek Woolfson

University of Bristol <https://orcid.org/0000-0002-0394-3202>

Article

Keywords:

Posted Date: September 10th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10344653/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Abstract

Programming three-dimensional order in peptide-based materials requires lateral interactions that are precise enough to propagate over hundreds of nanometres, yet robust enough to operate in water. Here, we show that a rationally designed coiled-coil peptide meets both demands by assembling into a hierarchical suprahelix that promotes spontaneous crystallisation under physiological buffer conditions. Coiled-coil dimers wind around one another to generate the higher-order suprahelix. Subtle lysine-to-arginine substitutions tune lateral cohesion, revealing a non-monotonic dependence on arginine density: 46-residue peptides with three or six arginine residues form ellipsoidal nanocrystals, whereas arginine-free or arginine-rich variants form amorphous aggregates. Time-resolved spectroscopy and cryogenic electron microscopy show that crystallisation proceeds by a non-classical pathway, in which condensed protofibrils align before consolidating into an ordered lattice. Cryo-electron tomography supports the single-domain nature of the resulting nanocrystals, and three-dimensional electron diffraction shows that both crystalline variants adopt the same near hexagonal unit-cell metrics. These findings translate a principle from protein crystallography, that arginine favours crystal contacts relative to lysine, into a sequence-level design parameter for coiled-coil nanocrystallization, providing a route to programmable three-dimensional order in soft molecular materials.

Introduction

Achieving rational control over three-dimensional (3D) order in molecular materials remains a central challenge in chemistry and materials science.^[1–6] In biological systems, this problem is solved through sequence-encoded interactions: the primary structure of a protein specifies the geometry, strength and periodicity of non-covalent forces that drive hierarchical self-assembly across multiple length scales.^[3–8] Cytoskeletal filaments, intracellular scaffolds and metabolic architectures exemplify how spatial order can emerge directly from chemical information encoded at the molecular level.^[4, 6, 9–14] These systems highlight a key principle for biomolecular materials design: structure can be programmed by sequence rather than arising as a passive consequence of bulk interactions.

In synthetic peptide systems, major advances have enabled the rational design of helices, oligomers, fibres and dynamic supramolecular assemblies.^[8, 12, 15–18] However, despite this progress, encoding reproducible 3D crystalline order in short peptides remains exceptionally difficult.^[2, 4, 11, 19–21] Lateral packing requires highly specific and geometrically regular interactions that must propagate coherently over hundreds of nanometres, such that even small deviations in side-chain orientation or charge distribution can destabilise long-range order. As a result, many peptide assemblies adopt anisotropic morphologies, such as fibres, tubes or sheets, while isotropic 3D crystals often require external triggers, including metal coordination, confinement or slow annealing, and can proceed through non-classical pathways involving condensed intermediates or protofibrillar alignment.^[2, 11, 18, 21–27]

Insights from protein crystallography offer a compelling conceptual framework for addressing this challenge. Surface-entropy reduction strategies have long shown that replacing flexible charged or polar

surface residues can improve crystallisability.^[20, 28–30] More recently, statistical analyses have revealed that arginine residues are strongly enriched at protein crystal packing interfaces, and that Lys-to-Arg substitutions can enhance crystallisation propensity with minimal impact on protein stability.^[28] The guanidinium group of arginine supports bidentate hydrogen bonding, cation– π interactions and highly directional electrostatics, suggesting that arginine may promote sequence-dependent lateral cohesion in ordered assemblies. However, whether such guanidinium-mediated interactions can be exploited to promote crystallisation in short, *de novo* peptides, whose assembly rules differ fundamentally from those of folded proteins, remains largely unexplored. Notably, isolated examples have shown that arginine placement can influence order in supramolecular peptide nanotubes, but a predictive design principle for metal-free 3D crystallization in short coiled-coil peptides is still lacking.^[3, 4, 6, 12, 22]

Here, we show that a 46-residue homodimeric coiled-coil peptide assembles into a hierarchical suprahelix that promotes the spontaneous formation of single-domain nanocrystals (CCNCs) under physiological buffer conditions. Within this architecture, systematic Lys-to-Arg substitutions act as a chemical tuning parameter, which we identify as a non-monotonic dependence on arginine density: intermediate arginine contents favour crystallisation, whereas the arginine-free and arginine-rich limits produce amorphous aggregates. Using time-resolved spectroscopy, cryogenic electron microscopy, cryo-electron tomography and three-dimensional electron diffraction, we follow the assembly process from early condensed intermediates to the crystalline lattice and delineate a non-classical pathway in which condensed protofibrils align before consolidating into an ordered lattice. Together, these findings identify arginine density as a sequence-level design parameter, operating through suprahelical coiled-coil geometry, for programming three-dimensional order in soft peptide materials.

Results

Designing lateral interactions in homodimeric coiled-coil peptides

We previously reported a self-assembling homodimeric coiled-coil peptide, DoNA-GN1, which forms extended and flexible fibres in aqueous solution.^[31] To test whether controlled lysine-to-arginine substitutions can tune lateral interactions sufficiently to promote three-dimensional crystallisation, we redesigned this scaffold to create well-defined and chemically differentiated interaction surfaces along the coiled-coil helices (Fig. 1). The homodimeric architecture ensures that supramolecular assembly is governed by sequence information rather than stoichiometric mixing, providing a direct link between molecular design and higher-order structure.

The resulting peptide architecture comprises three negatively charged heptads linked via a short GN spacer to three positively charged heptads (Table 1). This design follows established coiled-coil principles: hydrophobic residues occupy the a (Ile) and d (Leu) positions to drive dimerisation; alanine residues at the b and c positions promote helicity; and charged residues at the e and g positions

modulate interhelical electrostatics.^[12, 15] All N-terminal heptads contain glutamate residues, whereas the C-terminal heptads carry lysine or arginine residues to promote offset assembly through electrostatic complementarity.^[12, 31] In addition, single asparagine residues are placed at central a positions within each charged block to enforce slipped, parallel dimer formation between the oppositely charged segments.

To systematically probe whether arginine side chains can promote the lateral cohesion associated with crystallisation, we generated a series of peptides, CCNC-R0, CCNC-R3, CCNC-R6 and CCNC-R8, containing 0, 3, 6 or 8 lysine-to-arginine substitutions within the positively charged domain (Table 1). Substitutions were introduced at e, g and f positions, preserving the overall heptad architecture while progressively increasing the density of guanidinium functionalities capable, in principle, of bidentate hydrogen bonding, cation– π interactions and directional electrostatics. This design establishes a controlled series of arginine contents while maintaining an otherwise constant coiled-coil scaffold, allowing us to directly assess whether arginine density controls the emergence of single-domain peptide nanocrystals.

Table 1
Amino-acid sequences of CCNC coiled-coil peptides. Sequences of the homodimeric coiled-coil peptides CCNC-R0, CCNC-R3, CCNC-R6 and CCNC-R8, each comprising three negatively charged heptads linked to three positively charged heptads via a short GN spacer. Systematic lysine-to-arginine substitutions in the positively charged segment generate defined arginine contents (0, 3, 6 or 8 residues). All peptides were C-terminally amidated and flanked by glycine residues.

Peptide	Sequence
	- gabcdef gabcdef gabcdef -- gabcdef gabcdef gabcdef -
CCNC-R0	G EIAALEK ENAALEW EIAALEQ GN KIAALKY KNAALKK KIAALKK G
CCNC-R3	G EIAALEK ENAALEW EIAALEQ GN KIAALKY KNAALKK R IAAL RR G
CCNC-R6	G EIAALEK ENAALEW EIAALEQ GN KIAALKY R NAAL RR R IAAL RR G
CCNC-R8	G EIAALEK ENAALEW EIAALEQ GN R IAAL R Y R NAAL RR R IAAL RR G

Homodimeric peptides are helical and exhibit sequence-dependent supramolecular order

All peptides were synthesised by Fmoc solid-phase peptide synthesis, purified by reverse-phase HPLC and characterised by high-resolution electrospray ionization mass spectrometry (Supplementary Figs. 1–4). Upon dissolution in Milli-Q water and dilution into DPBS (25 μ M), all four peptides adopted α -helical secondary structures, as evidenced by characteristic minima at 208 nm and 222 nm in circular dichroism (CD) spectra (Fig. 2a). Thermal denaturation monitored at 222 nm yielded comparable melting temperatures across the series (35–42°C; Supplementary Fig. 6), indicating that lysine-to-arginine substitutions do not strongly perturb α -helicity or thermal stability under these conditions.

Despite these similar secondary structures, the peptides displayed markedly different supramolecular assembly behaviour. Cryogenic electron microscopy (cryo-EM) after 24 h incubation revealed that CCNC-R3 and CCNC-R6 formed well-defined, ellipsoidal nanocrystals, whereas CCNC-R0 and CCNC-R8 assembled into disordered aggregates lacking detectable crystalline order (Fig. 2d–f and Supplementary Figs. 11 and 12). Quantitative image analysis showed that CCNC-R3 nanocrystals had a median length of 265 nm (median absolute deviation, MAD, 175 nm) and a median width of 125 nm (MAD 75 nm). CCNC-R6 crystals were larger and more regular by this analysis, with median dimensions of 395 nm (MAD 170 nm) in length and 170 nm (MAD 60 nm) in width (Fig. 2b,c). In both cases, the nanocrystals displayed elongated morphologies with aspect ratios of approximately 2:1.

Cryo-electron tomography supported a single particle morphology for CCNC-R3 and CCNC-R6 assemblies, showing smooth, continuous particles without obvious grain boundaries or domain fusion (Supplementary Figs. 14 and 15). Dynamic light scattering (DLS) measurements further revealed a strong dependence of early-stage condensation on arginine content. CCNC-R3 had an apparent critical assembly concentration, or apparent CAC, of approximately 120 nM, whereas CCNC-R6 assembled at substantially lower concentrations (apparent CAC $\approx$ 46 nM; Supplementary Figs. 9 and 10), consistent with greater condensation propensity in the more arginine-rich crystallising variant.

Together, these observations reveal a non-monotonic dependence of crystallisability on arginine density. Peptides lacking arginine (CCNC-R0) did not form detectable ordered assemblies under these conditions, while excessive guanidinium content (CCNC-R8) could disrupt productive lateral packing, likely through electrostatic frustration or stabilisation of disordered intermediates. Only intermediate arginine contents (CCNC-R3 and CCNC-R6) support supramolecular ordering into single-domain peptide nanocrystals.

(a) Circular dichroism spectra of CCNC-R0, CCNC-R3, CCNC-R6 and CCNC-R8 measured at 25 μ M in DPBS, showing comparable α -helical secondary structures. **(b,c)** Width **(b)** and length **(c)** distributions of CCNC-R3 and CCNC-R6 nanocrystals after 24 h incubation ($n = 200$ particles per condition, from cryo-EM image analysis). **(d,e)** Representative cryo-EM micrographs of CCNC-R3 **(d)** and CCNC-R6 **(e)** nanocrystals after 24 h, showing ellipsoidal morphologies. Scale bar 200 nm. **(f)** Additional morphologies observed for CCNC-R3, including fragmented sheet-like assemblies. Scale bar 1 μ m.

Sequence-dependent access to non-classical crystallisation pathways

To elucidate how arginine content affects the temporal evolution of supramolecular order, we monitored assembly kinetics using CD spectroscopy, DLS, and time-resolved cryo-EM. CD spectra recorded over 24 h (DPBS, 25 μ M) remained unchanged for CCNC-R0, CCNC-R6 and CCNC-R8, indicating that α -helical secondary structure is established rapidly after dilution (Fig. 3a and Supplementary Fig. 7). In contrast, CCNC-R3 exhibited a progressive decrease in ellipticity at 222 nm, consistent with depletion of soluble material due to rapid crystallisation and sedimentation.

DLS measurements revealed pronounced sequence-dependent differences in assembly kinetics (Fig. 3b,c). CCNC-R3 formed assemblies with apparent hydrodynamic radii of approximately 100 nm immediately after dilution, followed by discontinuous growth to apparent radii of ~ 300 nm, consistent with rapid nucleation and consolidation. At higher concentrations (50 μ M), this process manifested as an accelerated increase in apparent hydrodynamic radius after approximately 90 min, consistent with the dense, grid-spanning crystalline material observed by cryo-EM. By contrast, CCNC-R6 exhibited slower, nearly linear growth with minimal concentration dependence without abrupt kinetic transitions, suggesting a gradual pathway in which protofibrils condense, align, and subsequently reorganise into a crystalline lattice. This behaviour is consistent with the higher arginine content of CCNC-R6 stabilising long-lived intermediates that require extended realignment prior to lattice consolidation.

Time-resolved cryo-EM captured snapshots consistent with these non-classical assembly pathways (Fig. 3e,f). Both CCNC-R3 and CCNC-R6 rapidly formed dense, condensed intermediates populated by short protofibrils at the earliest observed timepoints. For CCNC-R6, these protofibrils appeared disordered at early time points (0–15 min) before progressively aligning and transforming into ellipsoidal crystalline particles in representative fields by approximately 1 h. In contrast, CCNC-R3 displayed nascent crystalline domains at the earliest recorded time point, with well-formed crystals emerging within 5 min and approaching mature morphology within 2 h. Low-magnification cryo-EM supported these kinetics: CCNC-R3 showed extensive crystal coverage within 15 min, whereas CCNC-R6 samples remained largely devoid of crystals until longer incubation times (~ 4 h; Supplementary Figs. 16 and 17).

Together, these results reveal two distinct sequence-dependent routes through a non-classical crystallisation pathway. Intermediate arginine content (CCNC-R3) promotes rapid nucleation and comparatively polydisperse growth, whereas higher arginine content (CCNC-R6) slows lattice consolidation, enabling extended rearrangement and the formation of larger, more regular particles with single-domain crystallinity (Supplementary Fig. 18).

(a) Time-resolved CD spectra of CCNC-R3 showing progressive loss of soluble α -helical signal consistent with depletion of soluble material during crystallisation. **(b,c)** DLS kinetics of CCNC-R3 **(b)** and CCNC-R6 **(c)** at peptide concentrations of 10, 25 and 50 μ M, reported as apparent hydrodynamic radius (AHR). **(d)** Schematic illustration of the proposed non-classical assembly pathway, shown in full for CCNC-R6, involving condensation, protofibril formation, alignment and lattice consolidation. CCNC-R3 likely follows the same sequence of steps but traverses the intermediates on a much shorter timescale, so that aligned protofibrils and nascent crystals are observed near-simultaneously. **(e,f)** Time-resolved cryo-EM images of CCNC-R3 **(e)** and CCNC-R6 **(f)** showing sequence-dependent differences in crystallisation kinetics. Scale bars, 1 μ m.

Hierarchical ordering from molecular to mesoscale length scales

To determine how sequence-dependent interactions are associated with propagation into long-range order, we analysed individual CCNC-R3 and CCNC-R6 nanocrystals using cryo-electron tomography (cryo-

ET) and three-dimensional electron diffraction (3D ED). Cryo-ET reconstructions showed elongated individual particles (Supplementary Fig. 14 and Supplementary Video 1). Gold colloids used as fiducials adhered to the peptide surfaces, enabling tilt-series alignment and robust three-dimensional reconstruction. In the analysed tomograms, the particles showed smooth, continuous surfaces without obvious grain boundaries, domain fusion or fragmentation.

The observed morphological uniformity is consistent with a non-classical crystallisation pathway in which ordered nanocrystals emerge from a single condensed precursor rather than by extensive coalescence of preformed crystallites. This pathway, comprising condensation, protofibril formation and alignment prior to lattice consolidation, is consistent with the kinetic signatures observed by spectroscopy and cryo-EM provides a plausible route to the formation of single-domain crystalline particles across hundreds of nanometres.

Suprahelical organisation and lattice metrics of coiled-coil nanocrystals

Fourier transforms of cryo-EM projection images showed a dominant layer line at 175 Å, corresponding to a suprahelical pitch of 350 Å (Fig. 4a,e). The CCNC peptide designs contain two α -helical segments separated by a short GN linker and capped by terminal glycine residues. The N- and C-terminal helices are designed to interact heterotypically through electrostatic interactions to favour a slipped assembly (Fig. 1 and Table 1). Structural modelling of this architecture yields an overall length of approximately 85 Å for each folded monomer. This corresponds to roughly four coiled-coil dimers per suprahelical pitch (Fig. 4b). As a consequence, surface charge motifs, such as the arginine-rich C-terminal regions in CCNC-R6, are presented around the suprahelix at approximately 90° intervals. In combination with complementary acidic patches on the N-terminal segment, this geometry is compatible with lateral packing in a near-hexagonal lattice that could also be described by a pseudo-orthorhombic cell.

Fourier filtering of cryo-EM images enhanced signal-to-noise and indicated both the suprahelical pitch and a lateral spacing of ~ 42 Å between adjacent coiled-coil dimers, consistent with established coiled-coil dimensions (Fig. 4d).^[32] Using these parameters, we constructed a structural model comprising a 6 $\times$ 6 array of suprahelical coiled coils (Fig. 4c). Simulated projection images of this model reproduced the principal periodic features of the experimentally filtered data, supporting the proposed packing geometry (Fig. 4f).

(a) Representation of a single suprahelical repeat showing alternating coiled-coil strands (blue and magenta). **(b)** Molecular architecture from peptide chain to suprahelical coiled-coil assembly. A single peptide chain is shown in yellow, which dimerises to form a homodimeric coiled coil in magenta. **(c)** structural model of the assembled crystal, illustrating the lateral packing of strands into a higher-order suprahelical architecture. **(d)** Experimental projection cryo-EM image showing periodic density consistent with the filament structure **(e)** Fourier-filtered cryo-EM projection image. **(f)** Simulated projection image of the structural model.

3D ED-based lattice metrics

To determine unit-cell metrics and assess crystalline coherence, we collected 3D ED datasets from multiple individual nanocrystals of CCNC-R3 and CCNC-R6 (Supplementary Figs. 19–21; Supplementary Tables S1 and S2). Merging of multiple tilt-series datasets yielded substantial 3D reciprocal-space coverage for both samples, supporting unit-cell determination despite a moderate resolution limit of approximately 12.5 Å (Table 2). Indexing revealed two geometrically consistent descriptions: a hexagonal primitive lattice ($a \approx 51$ Å, $c \approx 168$ Å) and a pseudo-orthorhombic C-centred cell ($a \approx 88$ Å, $b \approx 50$ Å, $c \approx 169$ Å). These two descriptions are geometrically equivalent, representing the same near-hexagonal lattice indexed in different cells; the hexagonal solution provides the more natural physical description. The 3D ED parameters agree well with the cryo-EM measurements: the ~ 50 Å lattice spacing is broadly consistent with the ~ 42 Å row-to-row distance observed by Fourier filtering, and the ~ 168 Å repeat along the filament axis corresponds to half the 350 Å suprahelical pitch, as expected for a half-turn repeat of the two equivalent strands.

Importantly, CCNC-R3 and CCNC-R6 have the same unit-cell metrics within experimental uncertainty, indicating that arginine content modulates crystallisation kinetics, but does not appreciably alter the final lattice geometry. Diffraction patterns from individual nanocrystals display single, unsplit sets of Bragg reflections, supporting their single-crystal nature. Peak broadening is consistent with the mosaicity typical of soft-matter crystals, and we did not detect clear reflection splitting, twinning or polycrystallinity within the collected datasets.^[25, 33] Together, these results show that modest arginine enrichment not only promotes crystallisation but enables the formation of structurally coherent, single-domain peptide nanocrystals with well-defined unit-cell metrics.

Table 2

Unit-cell parameters of CCNC-R3 and CCNC-R6 nanocrystals determined by three-dimensional electron diffraction. - Merged 3D ED datasets were indexed using both a hexagonal primitive cell and a pseudo-orthorhombic C-centred cell. Data extend to approximately 12.5 Å resolution.

Crystal	Cell Type	a (Å)	b (Å)	c (Å)	Laue Class
CCNC-R3	Hexagonal Primitive	50.8 ± 0.2	50.8 ± 0.2	168.7 ± 0.7	6/m or 6/mmm
	Orthorhombic C-Centred	88.2 ± 0.4	50.1 ± 0.1	169.1 ± 0.7	mmm
CCNC-R6	Hexagonal Primitive	51.3 ± 0.1	51.3 ± 0.1	168.0 ± 0.4	6/m or 6/mmm
	Orthorhombic C-Centred	88.6 ± 0.1	50.5 ± 0.1	168.8 ± 0.4	mmm

Discussion

The data presented here show that subtle lysine-to-arginine substitutions in a *de novo* designed coiled-coil peptide tune a robust and reproducible pathway towards three-dimensional crystallisation. Such behaviour is notable because relatively short *de novo* peptides, particularly those lacking metal-binding motifs or extended β -structured backbones, more commonly form fibres, tubes, sheets or amorphous

aggregates than single-domain crystalline particles under aqueous conditions.^[20, 28, 29, 34–37] By showing how arginine density tunes crystallisation through a hierarchical suprahelical architecture, this work identifies a sequence-level design parameter that connects concepts from protein crystallography with *de novo* peptide materials design.

The non-monotonic dependence of crystallisability on arginine density reveals that guanidinium-mediated interactions can impose both enabling and limiting constraints on assembly. In the absence of arginine, early-stage condensation appears to be insufficient to direct lateral order, whereas excessive guanidinium content may disrupt productive packing through electrostatic frustration or stabilisation of disordered intermediates. Only intermediate arginine contents appear to provide the balance of directional hydrogen bonding, multivalent electrostatics and packing flexibility needed for protofibrils to align and anneal into single-domain crystals. This non-monotonic arginine-density regime underscores the importance of tuning sequence-level interaction strengths to influence the free-energy landscape of soft-matter crystallisation.

The combination of time-resolved spectroscopy, cryogenic electron microscopy, tomography and electron diffraction supports a non-classical crystallisation pathway involving condensation, fibrillar alignment and lattice consolidation. Such pathways are increasingly recognised in soft and biomolecular materials, but are less often followed across length scales, from the nanometre-scale organisation of coiled-coil dimers to the hundreds-of-nanometres dimensions of the mature nanocrystals. The ability of CCNC assemblies to reorganise into single-domain nanocrystals from disordered protofibrillar intermediates suggests an annealing capacity of the suprahelical coiled-coil scaffold, likely enabled by the mobility of these intermediates and the angular periodicity of surface charge motifs presented by the suprahelix.

More broadly, this work shows that principles governing the crystallisation of folded proteins can inform the design of short synthetic peptides by introducing appropriate interaction motifs at controlled densities. This study focuses on a single coiled-coil architecture, and the atomistic basis of the arginine effect has not been resolved here and extending this design principle to other scaffolds and confirming the underlying interactions are natural next steps. The modular heptad-repeat architecture of coiled coils provides a versatile platform for exploring how periodic charge patterns, hydrophobic spacing and superhelical geometry can be exploited to programme three-dimensional order in soft molecular materials. The sequence-encoded crystallisation strategy described here offers a route towards peptide-based nanocrystals and establishes a foundation for designing ordered soft-matter architectures with tuneable structure and function.

Declarations

Acknowledgements

P.R., D.Y.W.N., K.I.A., D.N.W., and T.W. gratefully acknowledge financial support from the Max Planck-Bristol Centre for Minimal Biology and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – project number 464588647 (SFB1551 R04). I.H. acknowledges support from the H2020 Marie Skłodowska-Curie Actions programme of the European Commission (ITN SUPERCOL, grant agreement 860914). M.S. acknowledges support from the H2020 Marie Skłodowska-Curie Actions programme of the European Commission (ITN NanED, grant agreement 956099). K.I.A. and D.N.W. acknowledge support from the BBSRC of the UK (BB/V004220/1), and D.N.W. acknowledges support from the Novo Nordisk Foundation for establishing the Center for Protein Design, Copenhagen (NNF25SA0105927). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

P.R., D.Y.W.N. and T.W. conceived the project, with K.I.A. and D.N.W. advising on sequence design. P.R. performed the synthesis. P.R. and Z.R. performed the CD analysis. P.R. performed DLS measurements with support from the polymer analytics facility. I.H. performed the cryo-EM measurements and discussed with I.L and K.L. I.H. and M.S. performed the 3D ED measurements with supervision from U.K. T.H.S. and D.N.W. helped analyse the structural data and T.H.S. created the coiled-coil model. P.R., I.H., D.Y.W.N., T.H.S. and T.W. wrote the manuscript. K.L., D.Y.W.N., D.N.W. and T.W. supervised the project.

Data Availability

The data that support the findings of this study are available in the supporting information and source data are available in the Zenodo repository.

References

1. Yan R, Hu Y, Liu F, Wei S, Fang D, Shuhendler AJ, Liu H, Chen HY, Ye D (2019) J Am Chem Soc 141:10331–10341
2. Merg AD, Touponse G, Van Genderen E, Blum TB, Zuo X, Bazrafshan A, Siaw HMH, McCanna A, Brian Dyer R, Salaita K, Abrahams JP, Conticello VP (2020) J Am Chem Soc 142:19956–19968
3. Wang F, Gnewou O, Modlin C, Beltran LC, Xu C, Su Z, Juneja P, Grigoryan G, Egelman EH, Conticello VP (2021) Nat Commun 12:407
4. Li Z, Wang S, Nattermann U, Bera AK, Borst AJ, Yaman MY, Bick MJ, Yang EC, Sheffler W, Lee B, Seifert S, Hura GL, Nguyen H, Kang A, Dalal R, Lubner JM, Hsia Y, Haddox H, Courbet A, Dowling Q, Miranda M, Favor A, Etemadi A, Edman NI, Yang W, Weidle C, Sankaran B, Negahdari B, Ross MB, Ginger DS, Baker D (2023) Nat Mater 22:1556–1563
5. Merg AD, Touponse G, van Genderen E, Zuo X, Bazrafshan A, Blum T, Hughes S, Salaita K, Abrahams JP, Conticello VP (2019) Angew Chemie - Int Ed 58:13507–13512
6. Egelman EH, Xu C, Dimaio F, Magnotti E, Modlin C, Yu X, Wright E, Baker D, Conticello VP (2015) *Structure* 23, 280–289

7. Burkhard P, Ivaninskii S, Lustig A (2002) *J Mol Biol* 318:901–910
8. Utterström J, Naeimipour S, Selegård R, Aili D (2021) *Adv Drug Deliv Rev* 170:26–43
9. Mellman I, Nelson WJ (2008) *Nat Rev Mol Cell Biol* 9:833–845
10. Hong Y, Najafi S, Casey T, Shea J, Han S-I, Hwang DS (2022) *Nat Commun* 13:7326
11. Yuan C, Ji W, Xing R, Li J, Gazit E, Yan X (2019) *Nat Rev Chem* 3:567–588
12. Wu Y, Collier JH (2017) *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 9:1–17
13. Bremer A, Farag M, Borchers WM, Peran I, Martin EW, Pappu RV, Mittag T (2022) *Nat Chem* 14:196–207
14. McCahill AL, Zhang T, Saven JG, Kloxin CJ, Pochan DJ (2024) *ACS Nano* 18:25695–25707
15. Woolfson DN (2023) *J Biol Chem* 299:104579
16. Hu J, Li J, Jiang J, Wang L, Roth J, McGuinness KN, Baum J, Dai W, Sun Y, Nanda V, Xu F (2022) *Nat Commun* 13. 10.1038/s41467-022-34127-6
17. Urbach ZJ, Marrufo N, Do QTH, Le JN, Wierzbicki M, Song Y, Mustafa K, Wang F, Egelman EH, Guan Z, Tobias DJ, Hochbaum AI (2025) 10.1021/jacs.5c05344
18. Tian Y, Polzer FB, Zhang HV, Kiick KL, Saven JG, Pochan DJ (2018) *Biomacromolecules* 19, 4286–4298
19. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI (2004) *Science* 303:1352–1355
20. Czepas J, Devedjiev Y, Krowarsch D, Derewenda U, Otlewski J, Derewenda ZS (2004) *Acta Crystallogr Sect D Biol Crystallogr* 60:275–280
21. Xu S, Zhang H, Qiao B, Wang Y (2021) *Cryst Growth Des* 21:7306–7325
22. Jorgensen MD, Chmielewski J (2022) *Chem Commun* 58:11625–11636
23. Fernandes R, Chowdhary S, Mikula N, Saleh N, Kanevche K, Berlepsch Hv, Hosogi N, Heberle J, Weber M, Böttcher C, Koks B (2022) *Angew Chem Int Ed* 61. 10.1002/anie.202208647
24. Hsieh MC, Lynn DG, Grover MA (2017) *J Phys Chem B* 121:7401–7411
25. Van Driessche AES, Van Gerven N, Joosten RRM, Ling WL, Bacia M, Sommerdijk N, Sleutel M (2021) *Nat Commun* 12:6–13
26. Nepal M, Sheedlo MJ, Das C, Chmielewski J (2016) *J Am Chem Soc* 138:11051–11057
27. Curtis RW, Nepal M, Nambiar M, Jorgensen MD, Chmielewski J (2025) *Pept Sci* 117:1–6
28. Banayan NE, Loughlin BJ, Singh S, Forouhar F, Lu G, Wong KH, Neky M, Hunt HS, Bateman LB, Tamez A, Handelman SK, Price WN, Hunt JF (2024) *Protein Sci* 33:1–23
29. Sokalingam S, Raghunathan G, Soundrarajan N, Lee SG (2012) *PLoS ONE* 7. 10.1371/journal.pone.0040410
30. Cooper DR, Boczek T, Grelewska K, Pinkowska M, Sikorska M, Zawadzki M, Derewenda Z (2007) *Acta Crystallogr Sect D Biol Crystallogr* 63:636–645

31. Boyle AL, Bromley EHC, Bartlett GJ, Sessions RB, Sharp TH, Williams CL, Curmi PMG, Forde NR, Linke H, Woolfson DN (2012) *J Am Chem Soc* 134:15457–15467
32. Sharp TH, Bruning M, Mantell J, Sessions RB, Thomson AR, Zaccai NR, Brady RL, Verkade P, Woolfson DN (2012) *Proc. Natl. Acad. Sci. U. S. A.* 109, 13266–13271
33. Elizebath D, Lim JH, Nishiyama Y, Vedhanarayanan B, Saeki A, Ogawa Y, Praveen VK (2024) *Small* 20, 1–9
34. Levy ED, De S, Teichmann SA (2012) 109, 10.1073/pnas.1209312109
35. Tesei G, Vazdar M, Jensen MR, Cagnell C, Mason PE, Heyda J, Skepö M, Jungwirth P, Lund M (2017) *Proc. Natl. Acad. Sci.* 114, 11428–11433
36. Maier R, Zocher G, Sauter A, Da Vela S, Matsarskaia O, Schweins R, Sztucki M, Zhang F, Stehle T, Schreiber F (2020) *Cryst Growth Des* 20:7951–7962
37. Kaygisiz K, Rauch-Wirth L, Dutta A, Yu X, Nagata Y, Bereau T, Münch J, Synatschke CV, Weil T (2023) *Nat Commun* 14. 10.1038/s41467-023-40663-6

Figures

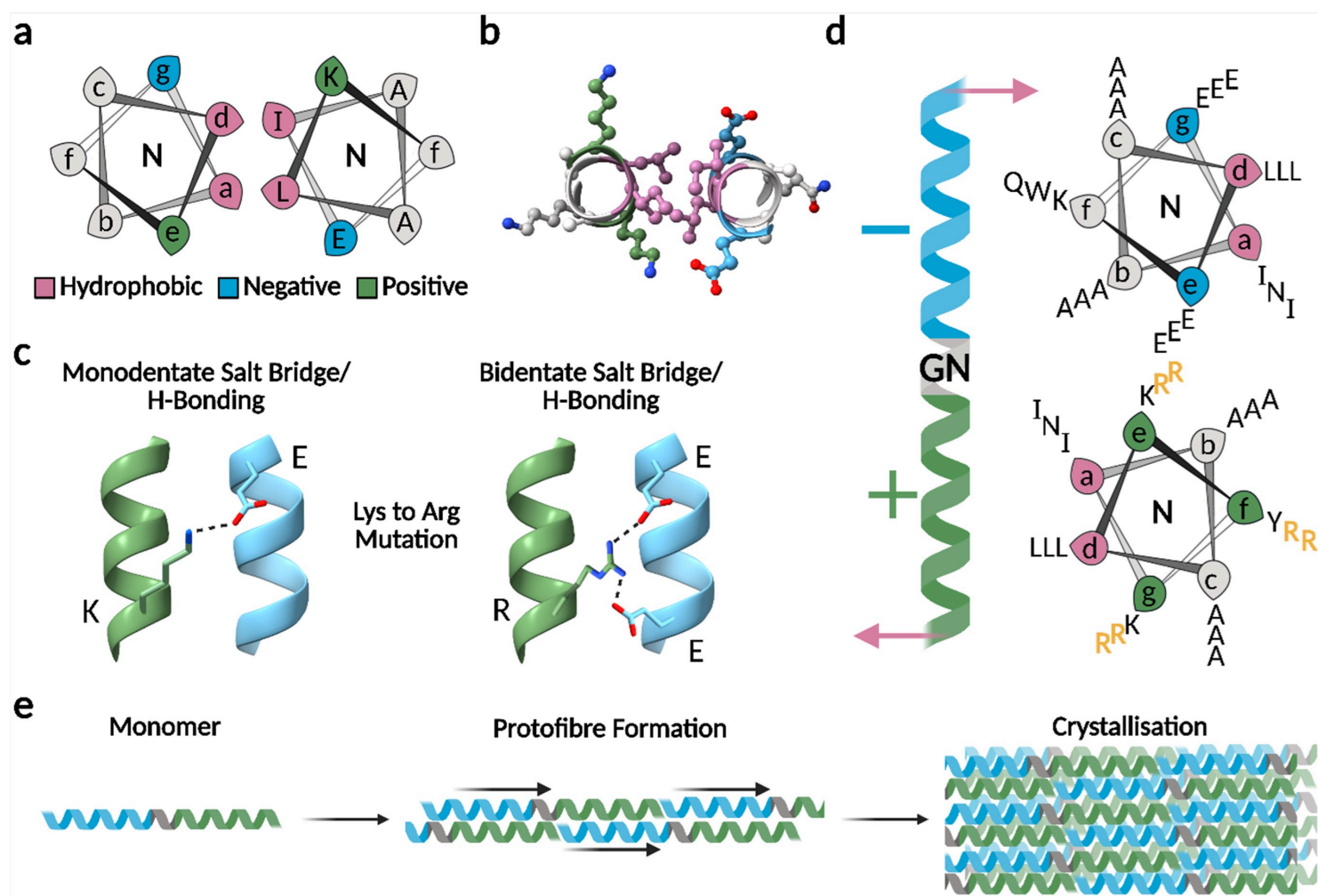


Figure 1

Sequence design and conceptual framework for arginine-tuned peptide crystallisation. (a) Helical wheel representation of the homodimeric coiled-coil heptad repeats (*gbcdefa*), highlighting hydrophobic *a/d* positions (pink), alanine-rich *b/c* positions (light grey), and charged *e/g* positions (blue for Glu, green for Lys/Arg). (b) Ball-stick representation of a negatively charged heptad (blue, right) interacting with a positively charged heptad (green, left) via knob-into-hole interactions (pink) and salt bridges. (c) Conceptual illustration of the differences between a Lys and Arg-containing basic site, highlighting the greater potential of arginine for multidentate hydrogen bonding and electrostatic interactions within a heptad. (d) Schematic representation of the CCNC architecture, comprising three negatively charged heptads (blue) linked to three positively charged heptads (green) via a GN linker (grey). Positions subjected to Lys to Arg mutation are highlighted in orange. (e) Illustration of the proposed assembly pathway from a peptide monomer via coiled-coil protofibrils into a crystal.

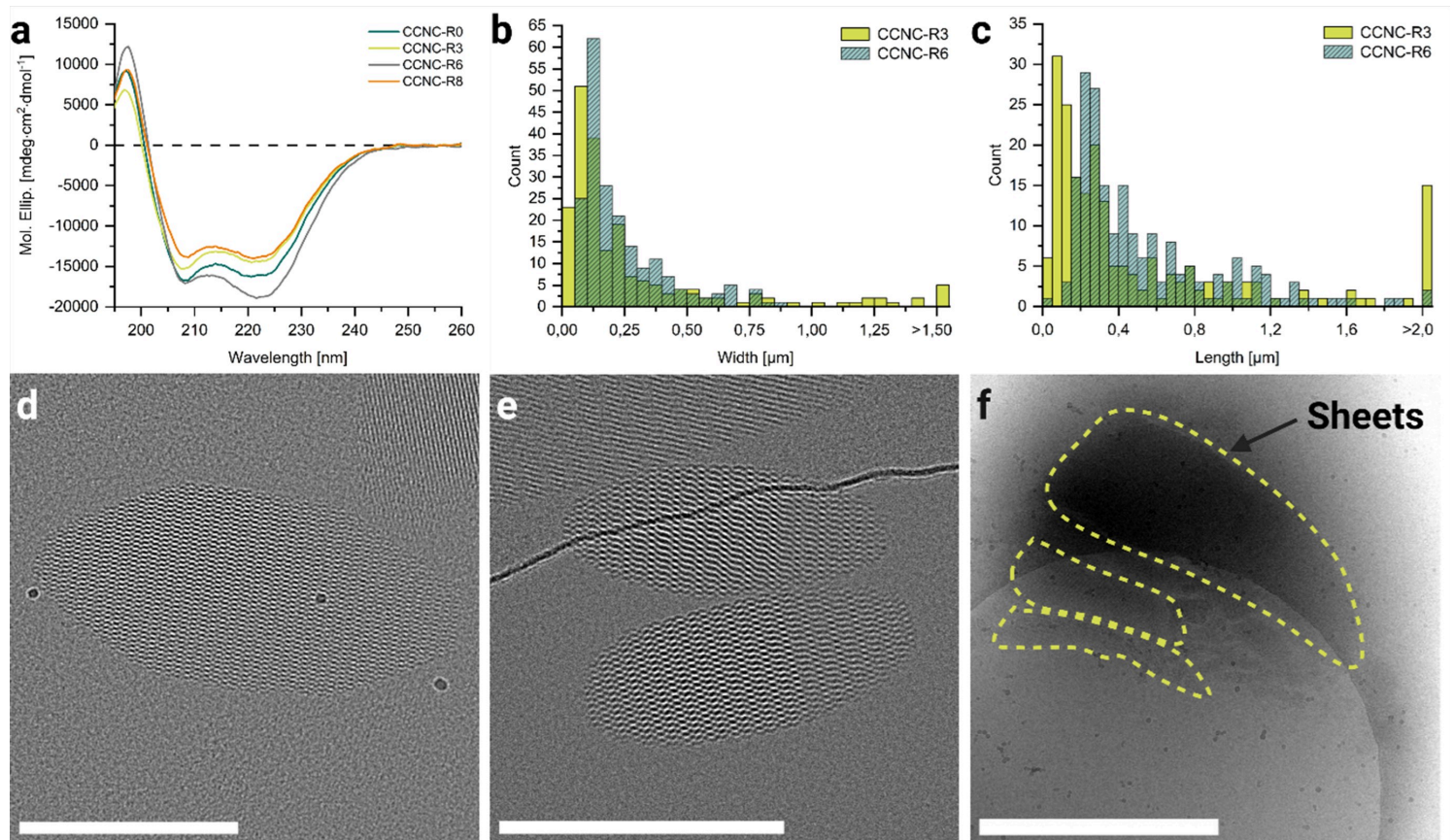


Figure 2

Solution-phase characterisation and crystallisation of CCNC coiled-coil assemblies.

(a) Circular dichroism spectra of CCNC-R0, CCNC-R3, CCNC-R6 and CCNC-R8 measured at 25 μ M in DPBS, showing comparable α -helical secondary structures. (b,c) Width (b) and length (c) distributions of CCNC-R3 and CCNC-R6 nanocrystals after 24 h incubation ($n = 200$ particles per condition, from cryo-EM image analysis). (d,e) Representative cryo-EM micrographs of CCNC-R3 (d) and CCNC-R6 (e) nanocrystals after 24 h, showing ellipsoidal morphologies. Scale bar 200 nm. (f) Additional morphologies observed for CCNC-R3, including fragmented sheet-like assemblies. Scale bar 1 μ m.

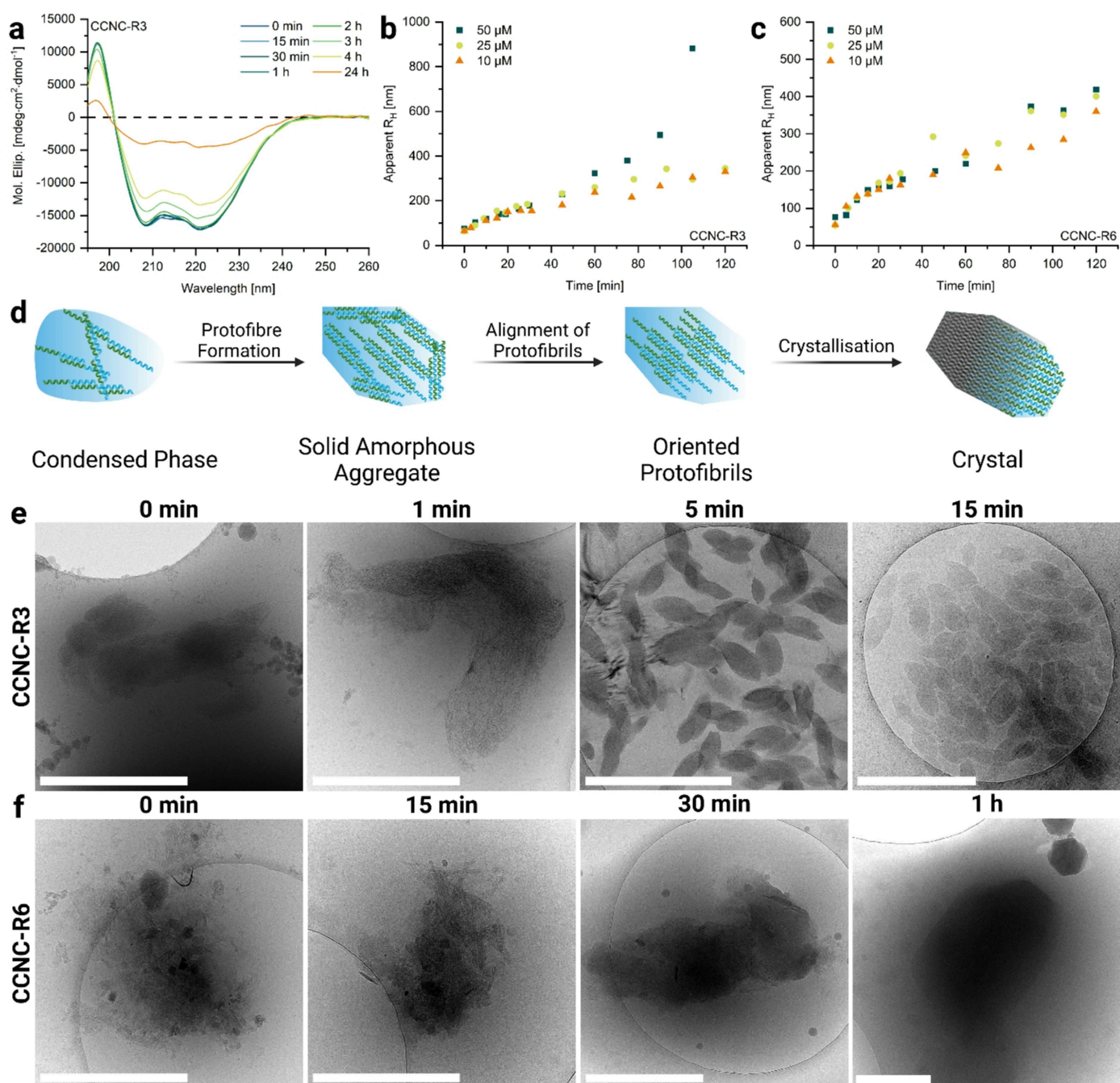


Figure 3

Sequence-dependent assembly kinetics and non-classical crystallisation pathways.

(a) Time-resolved CD spectra of CCNC-R3 showing progressive loss of soluble α -helical signal consistent with depletion of soluble material during crystallisation. **(b,c)** DLS kinetics of CCNC-R3 **(b)** and CCNC-R6 **(c)** at peptide concentrations of 10, 25 and 50 μ M, reported as apparent hydrodynamic radius (AHR). **(d)** Schematic illustration of the proposed non-classical assembly pathway, shown in full for CCNC-R6,

involving condensation, protofibril formation, alignment and lattice consolidation. CCNC-R3 likely follows the same sequence of steps but traverses the intermediates on a much shorter timescale, so that aligned protofibrils and nascent crystals are observed near-simultaneously. **(e,f)** Time-resolved cryo-EM images of CCNC-R3 **(e)** and CCNC-R6 **(f)** showing sequence-dependent differences in crystallisation kinetics. Scale bars, 1 μm .

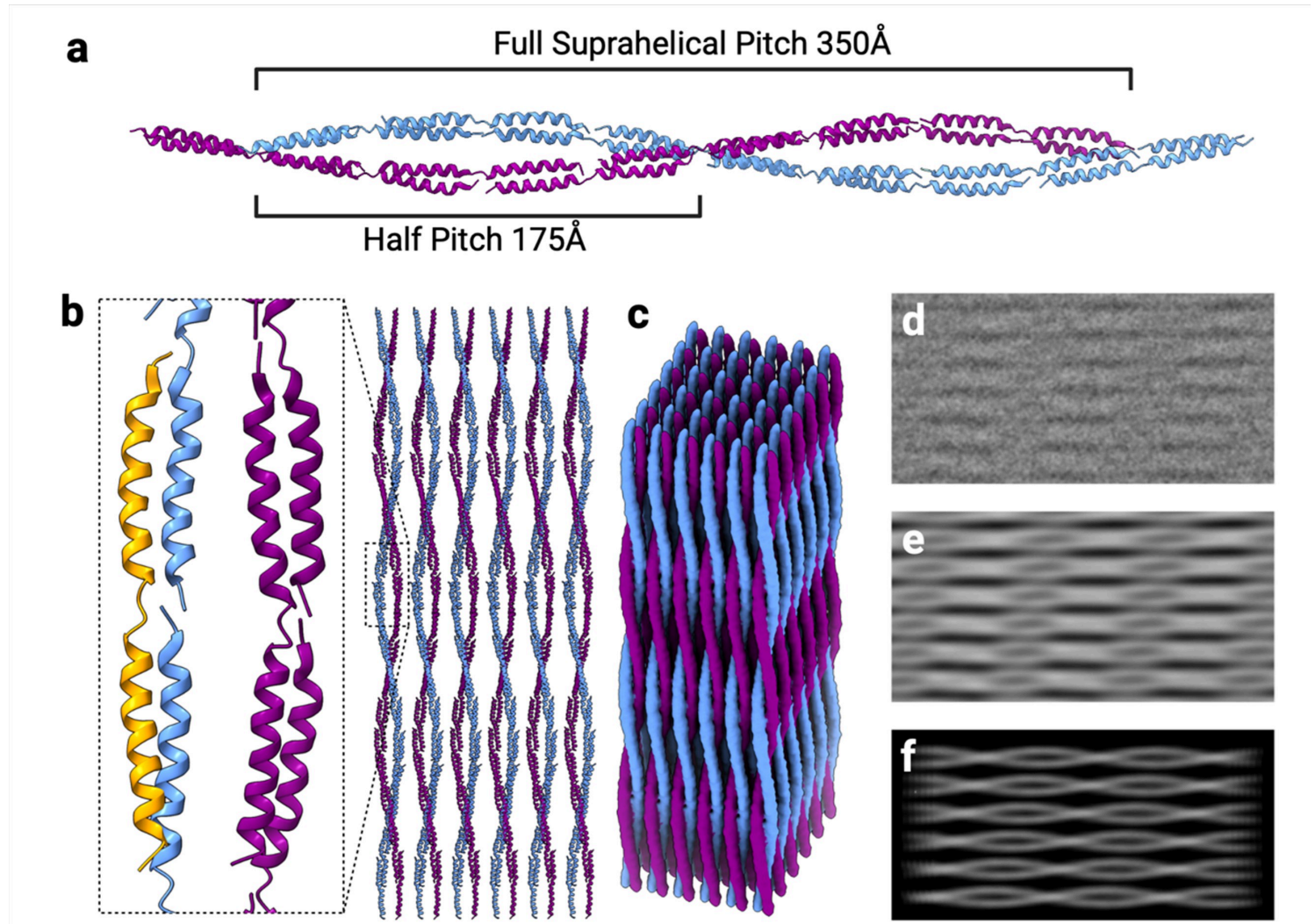


Figure 4

Hierarchical structural organisation and lattice metrics of CCNC nanocrystals.

(a) Representation of a single suprahelical repeat showing alternating coiled-coil strands (blue and magenta). **(b)** Molecular architecture from peptide chain to suprahelical coiled-coil assembly. A single peptide chain is shown in yellow, which dimerises to form a homodimeric coiled coil in magenta. **(c)** structural model of the assembled crystal, illustrating the lateral packing of strands into a higher-order suprahelical architecture. **(d)** Experimental projection cryo-EM image showing periodic density consistent with the filament structure **(e)** Fourier-filtered cryo-EM projection image. **(f)** Simulated projection image of the structural model.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [RothHarley2026SI.docx](#)
- [nrreportingsummary.pdf](#)