

π - π Stacking origin of irreversible dispersibility of graphene oxide

Received: 4 June 2025

Accepted: 9 March 2026

Published online: 27 March 2026



Yue Gao^{1,7}, Ya Wang^{1,2,7}, Yangchao Liao^{3,4,7}, Dan Chang¹, Li Peng¹, Ruimin Li⁵, Xiaoli Zhao⁵, Kaiwen Li¹, Hang Li¹, Min Cao^{1,2}, Peng Li¹, Yingjun Liu^{1,6}, Xin Ming¹✉, Zhiping Xu⁴✉, Chao Gao^{1,6} & Zhen Xu^{1,6}✉

Graphene oxide (GO) has become an increasingly important industrial chemical and useful two-dimensional material. The complexity in both functional groups and heterostructure of GO offers its rich chemistry yet complicates its stability, dispersibility and processing property. Interactions with functional groups have been pioneered to explain these confusing properties of GO. However, the critical role of structural heterogeneity keeps elusive. Here, we report that the irreversible dispersibility of GO solid origins from the interlayer π - π stacking and the accessibility between conjugated domains. Experiments and simulations reveal that the exclusion of interlayer water leads to irreversible π - π stacking. This insight into the π - π stacking mechanism informs the design of selective gelation paths, enabling the scalable, continuous production of highly conductive graphene-based hydrogel for neural probes. Our work unveils a general mechanism for confusing dispersibility of GO and opens supramolecular interactions modulating methods for assembled structures and materials.

Graphene oxide (GO) is an ancient but newly developing chemical, derived from graphite^{1–6}. Especially for recent decades, GO has become an important industrial chemical⁷ and two-dimensional material for wide applications, spanning from graphene products^{6–9}, optoelectronics⁶, energy storage⁸ to sensors⁶, environmental governance^{8,9} and biomedicines⁸. Despite being produced in large scale and widely used, the complexity of GO, both in its heterostructure and interactive behaviors, still remains to be clearly understood and precisely controlled, which determines its realistic productions, processing and applications^{10–13}. An important example is that GO can exist as single layers in water and other polar solvents as synthesized but dried GO solids cannot redisperse into single layers, by either mild stirring or violent ultrasonication^{14,15}. This puzzling behavior greatly constrains the stable storage, mass transport and fluid processing of GO.

Long-term researches have revealed that GO is nonstoichiometric in chemical composition and heterogeneous in molecular structure^{10,11}. As a newly developed consensus, GO is a two-dimensional giant molecule with a mosaic heterostructure, which is collaged by sp^3 -hybridized carbon domains decorating by dangling oxygen containing groups and sp^2 -hybridized carbon domains with extended π -electron delocalization¹¹. From the one complex side of functional groups, the spontaneous migration and transition contribute to the metastability of GO^{4,5}. The bonding interactions with other agents, such as metal ions¹⁴, or chemical self-crosslinking¹⁵ have been proposed to elucidate the puzzling dispersibility of GO. From the other complex side of mosaic heterostructure, GO exhibits the amphiphilic attribute as a 2D surfactant¹⁶, which is a close analogy of amphiphilic block polymers¹⁷. We reason that the complex mosaic heterostructure of GO should play

¹MOE Key Laboratory of Macromolecular Synthesis and Functionalization, International Research Center for X Polymers, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou, PR China. ²Center for Healthcare Materials, Shaoxing Institute, Zhejiang University, Shaoxing, PR China. ³School of Civil and Environment Engineering, Changsha University of Science and Technology, Changsha, PR China. ⁴Applied Mechanics Laboratory, Department of Engineering Mechanics and Center for Nano and Micro Mechanics, Tsinghua University, Beijing, PR China. ⁵School of Materials Science and Engineering, Tongji University, Shanghai, PR China. ⁶Shanxi-Zheda Institute of Advanced Materials and Chemical Engineering, Taiyuan, PR China. ⁷These authors contributed equally: Yue Gao, Ya Wang, Yangchao Liao. ✉ e-mail: xin_ming@zju.edu.cn; xuzp@tsinghua.edu.cn; zhenxu@zju.edu.cn

another crucial role in understanding its concerned dispersibility and assembly behaviors.

Here, we report that the interlayer π - π stacking acts as unneglectable origin of irreversible dispersibility of GO. We reveal that the dispersibility of GO solids is directly determined by the extent of π - π stacking, which can be enhanced by higher drying temperature, lower oxidation and deeper reduction. Spectrum track and molecular dynamic (MD) simulations verify the dominating role of π - π stacking in non-dispersible GO solids. Increasing oxidation degree and shielding π - π stacking achieve the reversible dispersion of GO solids. The π - π stacking origin guides selective gelation paths of GO and initiates the scalable preparation of highly conductive graphene-based hydrogels for implantable probes. Our work exhibits the evidenced contribution of π - π interaction in GO assembly materials and provides a supramolecular view to decipher complex behaviors of GO.

Results and Discussion

The general non-dispersibility of GO dried solids

Initially, we systematically examined the redispersible issue of the representative GO, which was synthesized by the prevailing modified Hummers' method to have a carbon to oxygen (C/O) ratio of 1.96

(Supplementary Fig. 6a). Usual dry methods, including lyophilizing at low temperature, ambient drying and vacuum drying at temperatures ranging from 20 to 100 °C, were adopted to prepare GO solids. After being redispersed in water, we found that all these dried solids cannot be completely dispersed and remained aggregates in different extents (Fig. 1a, Supplementary Fig. 7). Even as drying under mild conditions (20 °C, 1 atm), the related functional group reactions are difficult to cause permanent aggregation. This irreversible dispersibility is a general nature of dried GO solids regardless of drying conditions, suggesting the existence of alternative mechanism, besides ionic crosslinking and covalent self-crosslink^{14,15}.

To qualitatively evaluate the redispersed extent of GO solids, we proposed a redisperse ratio (α) parameter to measure the proportion of redispersed GO single layers (Supplementary Note 1, Supplementary Figs. 1, 2 and Supplementary Table 1, 2). Figure 1b shows that the lyophilizing dried GO solid also has a low α of ~20%. For ambient drying, α decreases as the drying temperature increases, down to 5% at 100 °C (Fig. 1b). The redispersed GO spontaneously separated into upper dilute and lower concentrated parts after settling for about 12 h (Fig. 1a, Supplementary Fig. 7). Detailed atomic force microscopy (AFM) examinations demonstrate that the upper part almost contains

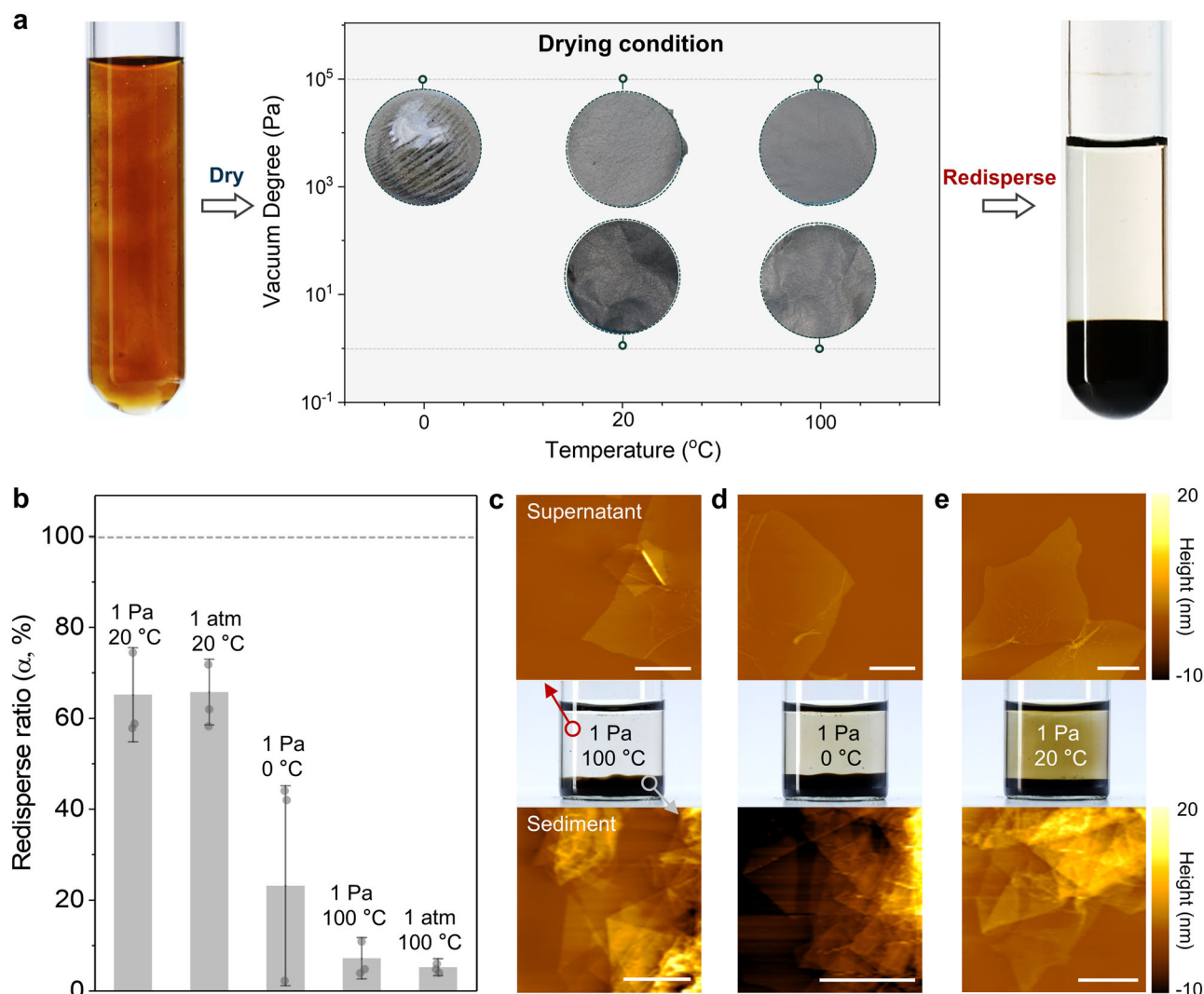


Fig. 1 | The irreversible dispersion behavior of GO solids. a The as-synthesized GO aqueous dispersion (photo, left), the ordinary GO solids dried by different conditions, categorized by drying vacuum degree and temperature (middle) and the unstable dispersion of GO solids precipitated and separated (photo, right). **b** The α of redispersed GO solid in water as related to the drying vacuum degree and

temperature. The data was expressed as mean $\pm$ standard deviation (SD) with a sample size of 3. **c–e** The dispersions of different GO solids (photo, middle) with GO single layer in supernatant (AFM image, top) and GO stack in sediment (AFM image, bottom). GO solids are dried at 100 °C (**c**), by lyophilization (**d**) and at 20 °C (**e**). Scale bars in **c, d** are 5 μ m.

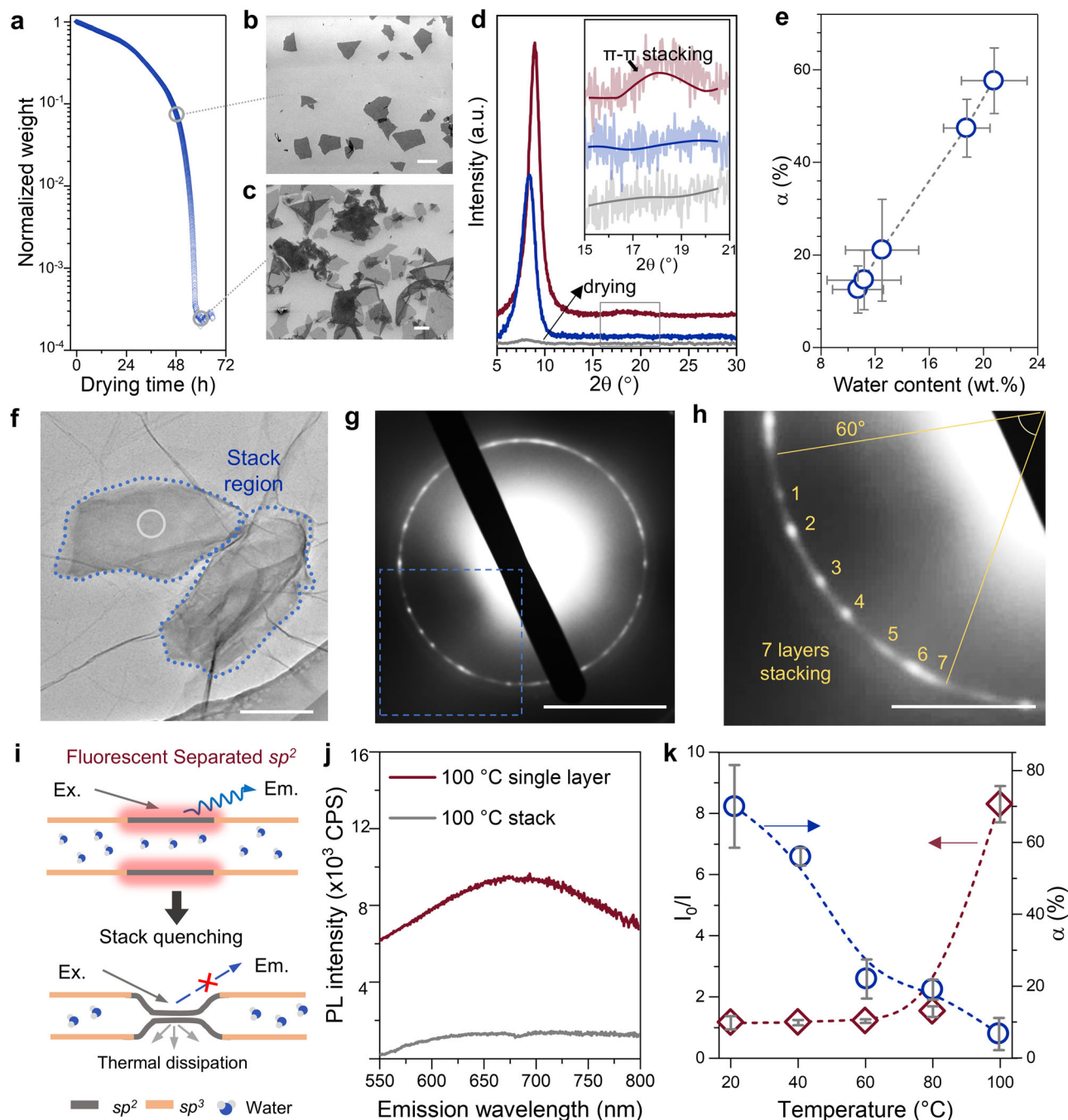


Fig. 2 | The occurrence of π - π stacking during drying process of GO dispersion.

a The weight tracking curve of drying process of GO dispersion. **b, c** Scanning electron microscopy (SEM) images of redispersed GO drying for 48 h (**b**) and 60 h (**c**) in **a**. Scale bars are 30 μm . **d** In-situ XRD patterns of GO dispersion during drying process. The inset is an enlarged view of the boxed region, showing the emerging of the peak at 18.6° . **e** The evolution of α with water content. The data were fitted using a linear model, yielding $R^2 = 0.9997$. **f** The TEM image of stacked GO precipitation in redispersed GO solid. Scale bar is 500 nm. **g, h** SEAD patterns of the

stacked region circled in **f**. Scale bars are 5 nm^{-1} **g** and 3 nm^{-1} **h**, respectively.

i Schematic illustration of quenching effect induced by π - π stacking. The fluorophores are the sp^2 region in the GO sheet. In GO solid, ACQ effect is triggered by π - π stacking of sp^2 regions. **j** The PL spectra of dispersions of heated single layer GO (in dispersion) and GO solid after the same 100°C heat treatment. The excitation wavelength is 350 nm. **k** The relationship of the ratio (PL intensity of heated GO single layer dispersion to redispersed GO solids) and α with heating temperature. The data was expressed as mean $\pm$ SD with a sample size of 3.

single-layered GO sheets with an average thickness of ~ 1 nm, while the lower part mainly contains stacked GO sheets with a thickness of 3–10 nm (Fig. 1c–e, Supplementary Fig. 7).

Tracking the occurrence of non-dispersible GO aggregates

We tracked the occurrence of irreversible dispersibility with decreasing water content at room temperature (Supplementary Note 2). Figure 2a shows that the water content or GO concentration (volume

ratio, ϕ) plays a key role to the irreversible dispersibility of GO. Once ϕ increases above $\sim 50\%$, non-dispersible GO aggregates began to appear (Fig. 2a–c, Supplementary Fig. 8). This phenomenon demonstrates that the irreversible stacking occurs as GO sheets become close enough to below ~ 1.3 nm (estimated by one-dimensional swelling model for ϕ of 50%, Supplementary Fig. 9)¹⁸.

To investigate the stacking mechanism during drying, we used in-situ X-ray diffraction (XRD) to track the structure evolution along

increasing ϕ . Dilute GO dispersion ($\phi \sim 0.5\%$) exhibited no diffraction peak and two diffraction 2θ peaks around 8° and 18.6° emerged in sequence during drying (Fig. 2d). The peak at 8° corresponds to an interlayer spacing of ~ 1 nm, while the latter peak at 18.6° corresponds to a narrower interlayer spacing of 0.47 nm, close to that of graphite (0.33 nm)¹⁹. Considering the effective distance of π - π interaction²⁰, this narrower interlayer spacing is consistent with dehydrated states involving π - π interactions between conjugated domains in GO sheets. When ϕ exceeds 50% , α quickly decreases from 60% to 12% , as the water content decreases from 22 to 10 wt% (Fig. 2e, Supplementary Fig. 10). These experimental track results clearly demonstrate that GO sheets spontaneously stack together as approaching close enough to form irreversible dispersed aggregates.

We examined the stacking structure of remaining GO aggregates by transmission electron microscopy (TEM). The morphology inspection and the selective area electron diffraction (SEAD) pattern reveal that GO sheets still partially remain conjugated lattice of graphene and disorderly stack together with a twisted interlayer configuration (Fig. 2f–h)^{21,22}. For example, seven stacked GO layers can be identified in aggregates (Fig. 2h). According to previous studies^{23,24}, such irregular stacking gives rise to a diffraction peak around 20° in XRD patterns (Fig. 2d). This observation illustrates the existence of conjugated domains in GO sheets and the corresponding irreversible stacking contributes to the poor redispersibility of GO solids.

To further confirm π - π stacking as the dominating reason in non-dispersible GO solids, photoluminescence (PL) analysis was conducted to compare the emission properties between individually dispersed GO sheets and GO stacks (Supplementary Note 3, Supplementary Figs. 3, 4 and Supplementary Tables 3, 4). Single layer GO sheets in water exhibited a PL emission peak around 675 nm under excitation at 350 nm, which denotes the isolated π domains in GO (Fig. 2i, j, Supplementary Fig. 11)^{25,26}. In contrast, for the same GO, the suspensions of its solids exhibited considerably decreasing PL intensity (Fig. 2j), which is a typical aggregation-caused quenching (ACQ) effect by π - π stacking between sp^2 domains^{27,28}. We quantified the PL intensity ratio (I_0/I) between single layer GO dispersions and solid dispersions identically heated at varied temperatures, observing enhanced quenching effect with increasing heat temperature²⁹. The enhanced π - π stacking intensifies this quenching effect and synchronously results a decreasing α of GO solids (Fig. 2k, Supplementary Figs. 12, 13), verifying the dominating role of π - π stacking for the redispersibility of GO solids.

π - π stacking mechanism of mosaic GO

The π - π stacking between GO sheets reasonably comes from its mosaic heterostructure. As a consensus, GO has demonstrated its structural complexity (Fig. 3a). To quantify its heterostructure, a parameter defined as the area fraction of sp^2 domains (β) was proposed (Fig. 3b). We examined the atomic structure of single layer GO sheets with different oxidation degrees (C/O ratio = 2.7 , 1.96 , 1.79 , Supplementary Fig. 6) by spherical aberration-corrected TEM (AC-TEM) and confirmed their mosaic heterostructure (Fig. 3c, Supplementary Fig. 14). As the oxidation degree increases, sp^3 domains become continuous and segment large sp^2 domains (> 10 nm²) into smaller ones (< 5 nm²), demonstrating a decrease in β from 63% to 34% . Regarding the redispersibility of GO solids, α decreases with the enlargement of β (Fig. 3d). Moreover, for different GO with diverse sources (different synthesis methods and batches), we can also find α of their solids decreases with increasing C/O ratio (increasing β), as shown in Supplementary Fig. 2. This direct correlation indicates that the existence of extended sp^2 domains is the structural origin of this irreversible interlayer π - π stacking, determining the irreversible dispersibility of GO solids.

We used MD analysis to investigate the formation mechanism of interlayer π - π stacking (Fig. 3e–h, Supplementary Information S2, Supplementary Figs. 35–45 and Supplementary Tables 5–9). Starting from two GO layers with intercalated water (initial interlayer spacing is

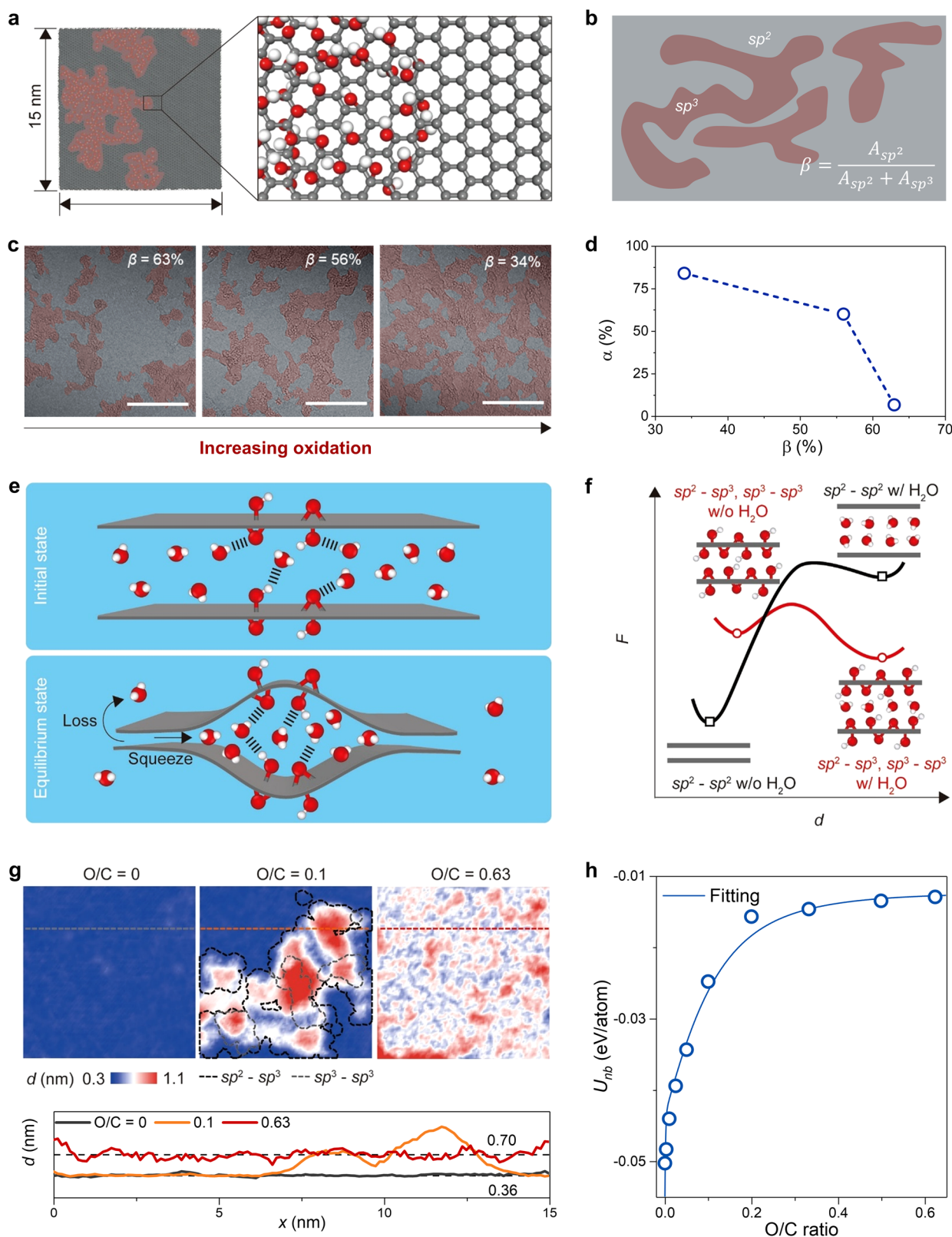
1 nm), sp^2 domains spontaneously get close to form stacking structures (Fig. 3e, Movie S1). At the same time, the intercalated water is squeezed out from the sp^2 - sp^2 stacking region and migrates to sp^3 regions with oxygen-containing groups¹⁰. This microscopic mechanism reveals the origin of occurrence of π - π stacking during drying as observed (Fig. 2). From the perspective of energy, the exclusion of interlayer water and maintenance of stacking in close proximity are favored in reducing the free energy of the sp^2 - sp^2 stacking region, while the sp^3 - sp^3 stacking region energetically tends to be separated by water (Fig. 3f). The competition between arrangement tendency of sp^2 domains and exfoliation tendency of sp^3 domains dominates the formation of stable π - π stacking. This critical competition generates a trend that weak oxidation or deep reduction deter the complete exfoliation to single layers and cause the irreversible dispersibility of solids, extending from pristine graphite to the usual GO^{10,30,31}.

To investigate the general trend of π - π stacking, we investigated the evolution of interlayer spacing (d) as varying O/C ratio of GO. Figure 3g shows that the average d expands from 0.36 nm to 0.70 nm as the O/C ratio increases to 0.63 . Despite expanding d , sp^2 domains consistently tend to get closer with the exclusion of water. In the case of O/C ratio of 0.1 , the local d of sp^2 - sp^2 stacking regions is around 0.36 nm, while the average d of other stacking regions is 0.81 nm with water intercalation. We further quantified the water-GO hydrogen-bond network in these oxygenated regions (Supplementary Figs. 38–40), which was reported to contribute to interlayer cohesion and mechanical response in GO dry solid materials and other lamellar solid materials^{32–36}. When the O/C ratio increases, the water-mediated hydrogen bonds become more numerous, since the local sp^2 - sp^2 contact is replaced by sp^3 - sp^3 contact (Supplementary Fig. 37). However, the non-bonding potential energy density (U_{nb}) of the GO bilayer rises monotonically with the O/C ratio (Fig. 3h and Supplementary Fig. 43), indicating that the formation of sp^3 - sp^3 contact (more hydrogen bonds) is energetically less favorable than the formation of sp^2 - sp^2 contact (more π - π stacking). The dependence of U_{nb} on the O/C ratio highlights that local sp^2 - sp^2 contact formation provides a strong stabilizing contribution, consistent with the irreversible stacking observed in our experiment (Fig. 3d). We note that the present models still idealize GO as sheets of finite, fixed geometries and do not explicitly represent full experimental polydispersity and edge-chemistry diversity; however, the key mechanistic trends persist across the tested O/C range, oxygen-pattern variants, and sheet sizes (Supplementary Figs. 38–45).

Combined with simulation and experiments, a clear aggregation mechanism can be demonstrated, that approaching sp^2 regions in mosaic GO are larger enough to form irreversible interlayer π - π stacking during drying. Although affected by functional groups, the extensive sp^2 regions (dozens to several hundred square nanometers) ensure sufficient dispersive interaction between π electrons of opposing conjugated structures to maintain a stable stacking structure^{20,37}. The significant role of π - π interactions serves as an evidenced contributor in assemble process of GO and graphene materials and can extend to functional graphitic nanomaterials, such as carbon nanotubes and nanodots.

Modulating π - π stacking for dispersible GO solids

Considering the π - π stacking origin, two strategies were proposed to enable the reversible dispersion of GO solids (Fig. 4a), including shielding π - π stacking by surfactants (Strategy 1, S1) and enhancing the oxidation degree of GO (Strategy 2, S2). We selected several usual anionic surfactants, such as sodium poly (styrene sulfonate) (PSS), sodium cholate (SC) and sodium dodecyl benzene sulfonate (SDBS), to shield the π - π stacking tendency of GO sheets³⁸. The α of dried GO solids with surfactants increases with surfactant content, reaching nearly 100% at surfactant content exceeding 40 wt% (Fig. 4b, Supplementary Fig. 15). The shielding of π - π stacking by surfactants is



evidenced by the widening interlayer spacing in GO/PSS solids and the disappearing π - π stacking peak in the XRD patterns (Supplementary Fig. 16). As a comparison, nonionic additives like polyvinyl pyrrolidone (PVP) only achieved α around 60% (Fig. 4b, Supplementary Fig. 17) for their weaker interaction with sp^2 domains³⁹. For the strategy 2, enhancing the intrinsic oxidation degree enlarges the sp^3 domain to strengthen the repulsion and reduce the accessibility between sp^2

domains. In experiments, GO solid completely redispersed to form single layers when the C/O ratio is below 1.77 (Fig. 4c, Supplementary Figs. 6, 18). For GO with high oxidation degree ($C/O < 1.77$), abundant functional groups provide numerous interaction sites for the formation of hydrogen bond and functional group reactions. Nevertheless, the improvement in re-dispersibility of GO solids can be observed at high oxidation, consistent with our simulation results (Fig. 3h). These

Fig. 3 | Molecular mechanism of π - π stacking between GO sheets. **a** Molecular models of GO with coexisting sp^2 and sp^3 regions, where sp^2 (blue) and sp^3 (red) regions are coded with pseudo colors for clear view. **b** The structural parameter (β) assessing the mosaic structure of GO. **c** AC-TEM images of GO single layers with different oxidation degrees. Scale bars are 10 nm. **d** The relationship between the redispersion ratio α and β measured in our experiments. **e** Schematic illustration of spontaneous π - π stacking process. During drying, interlayer water was squeezed towards hydrophilic sp^3 surface regions and preferentially coordinated via hydrogen bonding with oxygen-containing groups, thereby maintaining hydration in sp^3 -rich regions, while the sp^2 - sp^2 contact region becomes dehydrated and enables local

π - π stacking. **f** Diagrams of free energies, F , for graphene and GO bilayers, plotted as a function of interlayer spacings, d . The data points are obtained from MD simulations and schematic curves are added for illustration. **g** Interlayer spacing distributions of sp^2 - sp^2 , sp^2 - sp^3 regions, π - π (sp^2 - sp^2) stacking in stacked GO sheets with different O/C ratios, extracted from the equilibrated structures in MD simulations (top). Line scans of d corresponding to the dash lines in the top snapshots (bottom). **h** Evolution of non-bonding potential energy density U_{nb} between stacked GO sheets with O/C ratio. The data were fitted using a multi-exponential combination model, yielding $R^2 = 0.9718$.

results indicated that once the interlayer π - π stacking was effectively blocked, the re-dispersibility of GO solids could be improved.

Based on the both strategies, we prepared two GO dried powders that could be redispersed into single layers (Supplementary Fig. 19). As a prototype, two soluble GO powders were cyclically utilized to prepare continuous GO films (Fig. 4d, e, Supplementary Fig. 20). The recycled GO films exhibited comparative mechanical and electrical properties to the stock GO films (Fig. 4f–h). We also evaluated the production cost per kilogram of two kinds of GO powder for industrial production in future (Supplementary Fig. 21). The total raw material cost for both strategies is comparable, with only a marginal increase over the original cost of GO.

π -stack GO hydrogels

The revealed π - π stacking origin provides an alternative viewpoint to understand the assembly behaviors of GO stemming from its heterostructure. As immersed in water, GO solid film swelled into a gel state (Fig. 5a). Swollen GO gels kept structural integrity through the intrinsic π - π stacking crosslinks, as demonstrated by the lamellar structure with binary scattering peaks in XRD spectra (Fig. 5b, c). We termed this stable GO gel as π -stack gel, in analogy with assembled gels of block polymers, reflecting the similar intramolecular heterogeneous interaction mechanism⁴⁷. Confocal laser scanning microscopy (CLSM) and AFM inspections reveal the GO π -stack gel has a porous network of aligned GO laminates with thickness at sub-micrometer scale (Fig. 5d–f). In the theory frame of gel network^{40,41}, the equilibrium swelling ratio of this π -stack gel can be controlled by the π - π stacking tendency of GO (Supplementary Note 4 and Supplementary Fig. 5). By tuning aging time at room temperature and raising heating temperature, the equilibrium swelling ratio of GO gel was adjusted in a very broad range from 1.2 to nearly 600, corresponding to a solid content range of GO from 83% to 0.18% (Fig. 5g–i, Supplementary Figs. 22, 23). The π -stack gel state of dried GO solids gives an additional molecular mechanism for the reversible fusion and fission behavior of GO fibers⁴². In these processes, the irreversible molecular interface keeps stable for the π - π stacking network at swollen fiber surface (Supplementary Fig. 24).

Continuous preparation of graphene-based hydrogels

The unveiled influence of heterostructure of GO guides the successful large-scale preparation of chemical reduced GO (rGO) hydrogels with high electrical conductivity. In principle, the heterostructure of mosaic GO leads to two selective gelation strategies, that are tuning π - π stacking and crosslinking oxygen-containing groups. From the swollen GO π -stack gel, direct chemical reduction caused severe collapse of network with a large volume shrinkage over 50% (Fig. 5k), because of an increasing in crosslinking density induced by severe stacking of enlarged sp^2 domains (Fig. 3e–h, Fig. 5j). This direct reduction strategy failed the porous graphene-based network with large-scale preparation for multifunctional composite hydrogel with structural smoothness.

To fully exploit the selective interaction of GO, we designed a selective path to continuously prepare conductive rGO hydrogels. In this path, we proposed two sequencing interaction modulations: ion crosslinking to fix the network, followed by chemical reduction

(Fig. 5k). By these sequencing processes, the volume retention ratio of GO swollen gels reached 90% (Fig. 5j), enabling the wide range of rGO content in hydrogels and continuous preparation of meter-long rGO hydrogels with flat morphology (Fig. 6a, b). The ions crosslinking (e.g., Ca^{2+}) before reduction formed direct crosslink sites between sp^3 domains of GO sheets. This pre-formed Ca^{2+} crosslinked network depressed the direct stacking of GO sheets during reduction, thereby avoiding the severe collapse of network (Fig. 5j, k). Such a regulable and multi-level structural network is an ideal precursor for porous aerogel and hydrogel materials.

We realized the continuous preparation of large-area, uniform and soft rGO composite hydrogel film (RGHF) with high electrical conductivity, which is difficult to be achieved by the traditional blending method (Fig. 6a, Supplementary Figs. 25, 26). The RGHF exhibited both water stability and high conductivity of 55 S m^{-1} (Fig. 6b, Supplementary Fig. 27). The sequence gelation design ensures the excellent electrical conductivity, outperforming blending (10 S m^{-1}) and previous hydrothermal graphene-based hydrogels (3.2 S m^{-1})⁴³. Even after aging for 4 weeks, the mechanical performance of RGHF remains stable (Supplementary Fig. 28). Furthermore, the RGHF exhibited high processing compatibility, extending from continuous production with commercial GO films to refined micro-patterns with the feature size of 400 μm (Fig. 6b).

The scalable RGHFs allow the reliable fabrication of soft implantable hydrogel probes for in vivo neuromodulation (Fig. 6c), which is becoming a cutting-edge technique for merging brain-machine interfaces⁴⁴. We fabricated two proof-of-concept RGHE electrodes (RGHE) for in vivo neural stimulation and recording in a rat model (Supplementary Fig. 29). The RGHE exhibited a low cytotoxicity and excellent antibiofouling ability and biocompatibility, which laying the material foundation for it to be used as long-term implantable bioelectronics (Supplementary Figs. 30–33)⁴⁵. Following the connector assembly, the four-channel recording RGHE (100 μm diameter) was stereotactically implanted into the rat primary somatosensory cortex (coordinate: +3.4 mm AP; +3.8 mm ML; +1.15 mm DV, Fig. 6d, top). The soft RGHE demonstrated stable acquisition of continuous neural activities in a freely moving mouse (Fig. 6d, bottom), maintaining high-resolution recordings of the local field potential (LFP; at 1 kHz) and the extracellular action potential (AP; at 40 kHz, Fig. 6e) across all channels after two weeks implantation (Fig. 6e)⁴⁶. Then, the soft RGHE was conducted long-term neural recording for 4 weeks, and negligible performance degradation was observed, attributed to its great biocompatibility and stable electrode-tissue interface (Supplementary Fig. 34). The other paired stimulating RGHEs were implanted into the sciatic nerve of a rat to activate the ankle joint movements (Fig. 6f). Electrical stimulation using rectangular voltage pulses (200 mV, 1 Hz) induced the controlled ankle joint movements with wide angle ranging from 0° to 55° (Fig. 6f). Compared with the commercial rigid Au electrodes, the soft RGHEs achieved more efficient neuromuscular activation even at lower stimulation voltage of 40 mV (Fig. 6g), suggesting that the RGHE is a promising material candidate for bioelectronic interfaces^{47,48}.

In summary, we unveiled the interlayer π - π stacking as the primary mechanism of irreversible dispersibility of GO. The

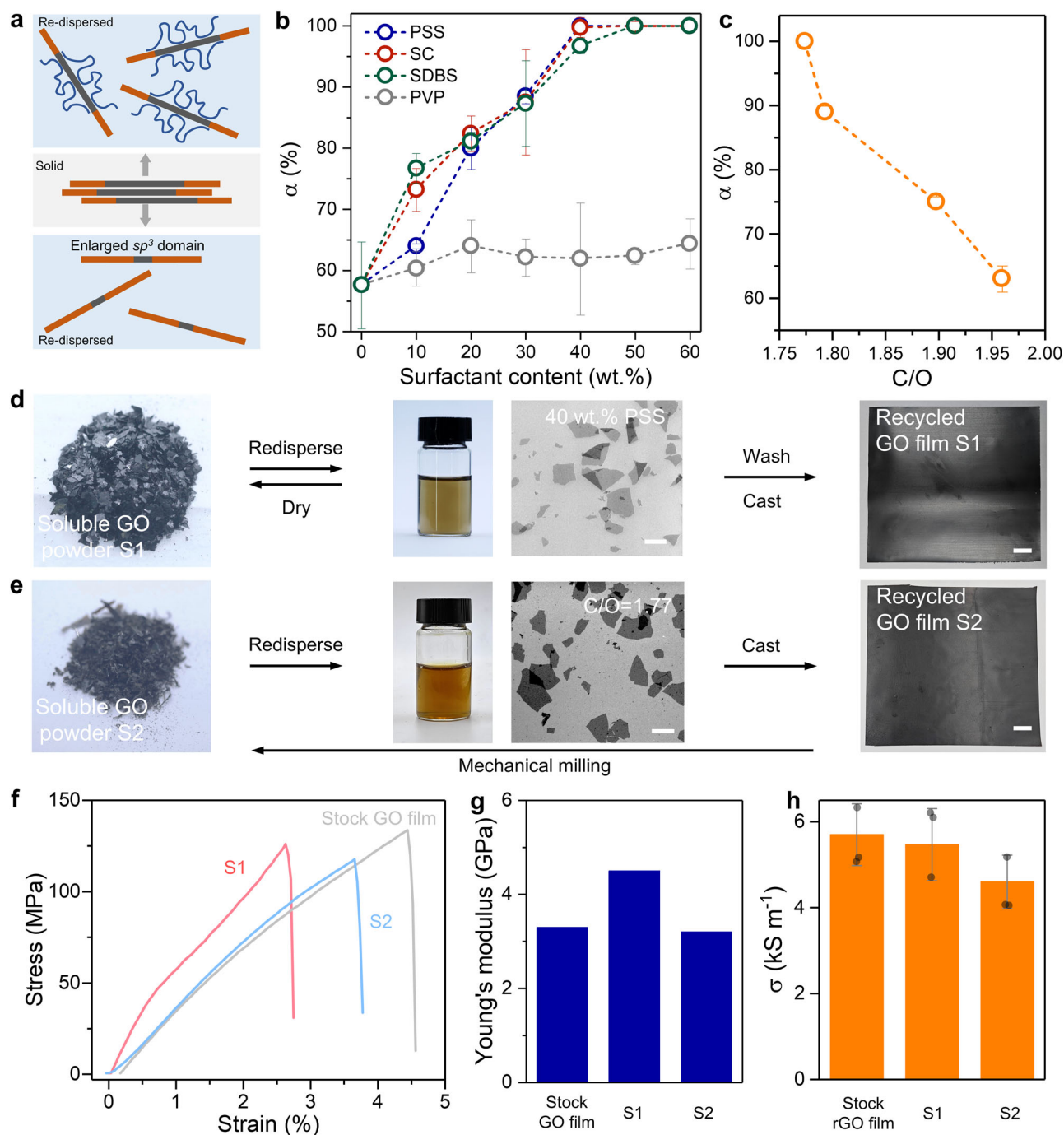


Fig. 4 | Improving the re-dispersibility of GO by the regulation of interlayer π - π interaction. **a** Schematic illustration of two proposed strategies to realize the redispersion of GO solids by blocking π - π stacking. **b** α changes with the surfactant contents in GO solid. **c** α changes with C/O of GO solid. **d** The preparation process of recycled GO film S1. Scale bar in the SEM image is 20 μm . The scale bar in the photo is 20 mm. **e** The preparation process of recycled GO film S2. Scale bar in the

SEM image is 20 μm . Scale bar in the photo is 20 mm. **f** Tensile curves of recycled GO film S1, S2 and stock GO film, respectively. **g** The Young's modulus of recycled GO film S1, S2 and stock GO film, respectively. **h** The electrical conductivity of recycled GO film S1, S2 and stock GO film, respectively. The data was expressed as mean $\pm$ SD with a sample size of 3.

heterogeneous mosaic structure of GO intrinsically contributes to the irreversible interlayer π - π stacking. This unveiled π - π stacking offers an alternative perspective to understand complex behaviors of GO from stability, dispersibility to processing properties. We proposed shielding π - π stacking and enhancing the oxidation degree, to relieve the π - π stacking tendency and achieve the complete dispersibility of GO dried solids. The π - π stacking origin guides the design of selective interactions to regulate the gelation paths for graphene-based hydrogels. We realized the large-scale production of highly

conductive rGO hydrogels for implantable neural electrodes. This π - π stacking effect of GO supplements a supramolecular view for understanding the complicated behaviors and enables precise bottom-up designs for delicate structures and materials of GO and other carbon nanomaterials.

Methods

Details of experiments and simulations are provided in Supplementary Information.

Preparation of GO solids drying by different methods

Five commonly used drying methods to prepare GO solids: air drying (20 °C, 1 atm), vacuum drying (20 °C, 1 Pa), lyophilization (0 °C, 1 Pa),

heating (100 °C, 1 atm) and vacuum heating (100 °C, 1 Pa). The GO dispersions were dried until no change in weight can be detected by an electronic balance (ME104, Mettler Toledo).

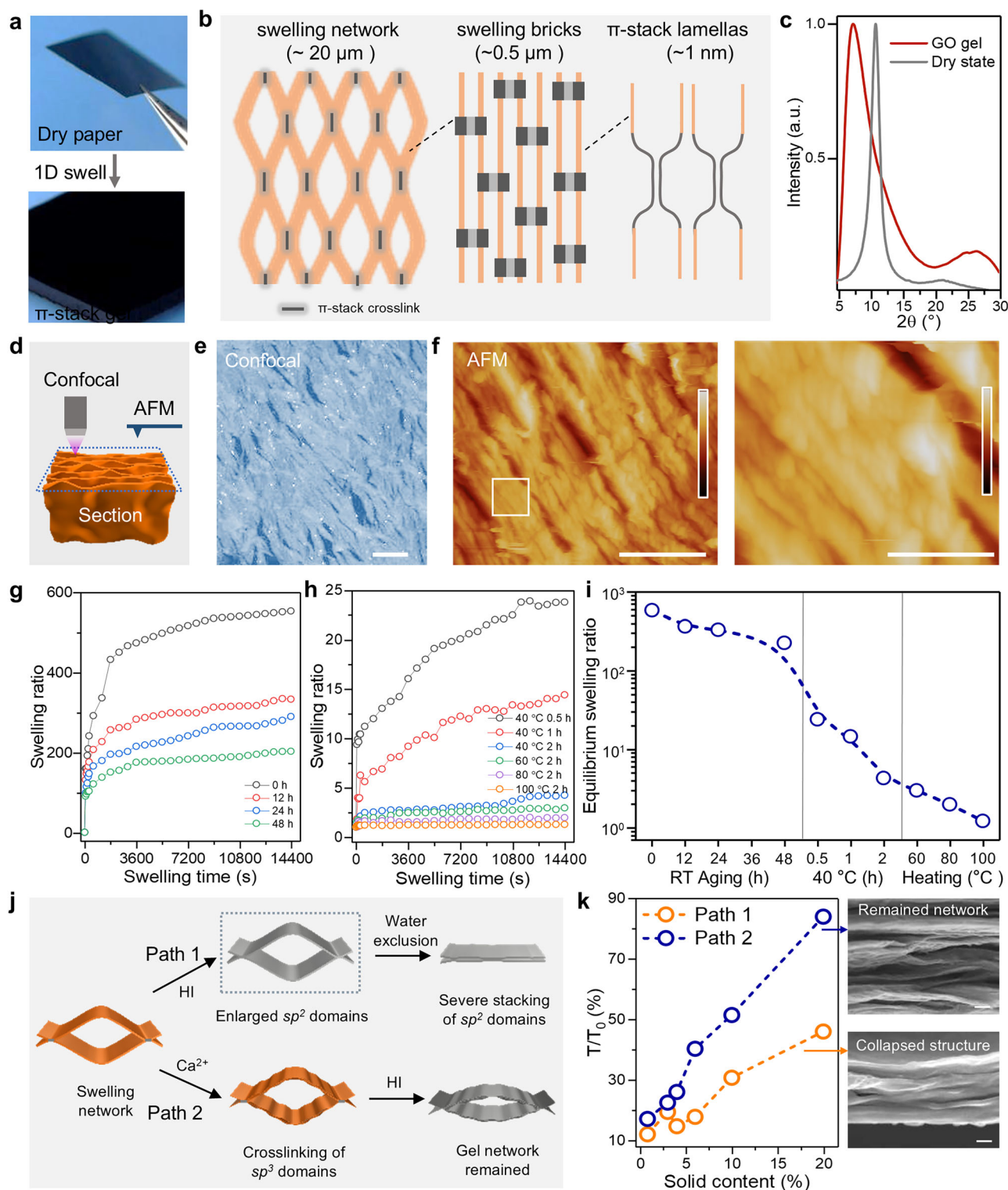


Fig. 5 | The GO gel caused by π - π stacking. **a** The 1D swelling of GO gel. Photo image of a piece of dry GO film (up) and the π -stack gel of GO (bottom). **b** Schematic illustration of the multi-scale structure of GO gel. **c** XRD patterns of dry state GO film and GO gel, respectively. **d** Schematic illustration of the sectional characterization of GO gel. **e** The CLSM image of GO gel. Scale bar is 40 μm. **f** The liquid phase AFM image of GO gel. Scale bar is 5 μm. The gradient scale is from -10 nm to 20 nm. The enlarged view of white box area (right). Scale bar is 1 μm. The gradient scale is from -10 nm to

20 nm. **g** The swelling curve of GO solids with different aging time. **h** The swelling curve of GO solids under different treatments. **i** The equilibrium swelling ratio of GO solids under different treatments. **j** Schematic illustration of the two gelation pathways based on π stack gels of GO. **k** The volume shrinkage ratio of GO gel with different solid contents after HI reduction of. The network structures of rGO gel prepared through Path 1 (dense structure, right bottom) and Path 2 (porous structure, right top), respectively. Scale bars are 100 nm.

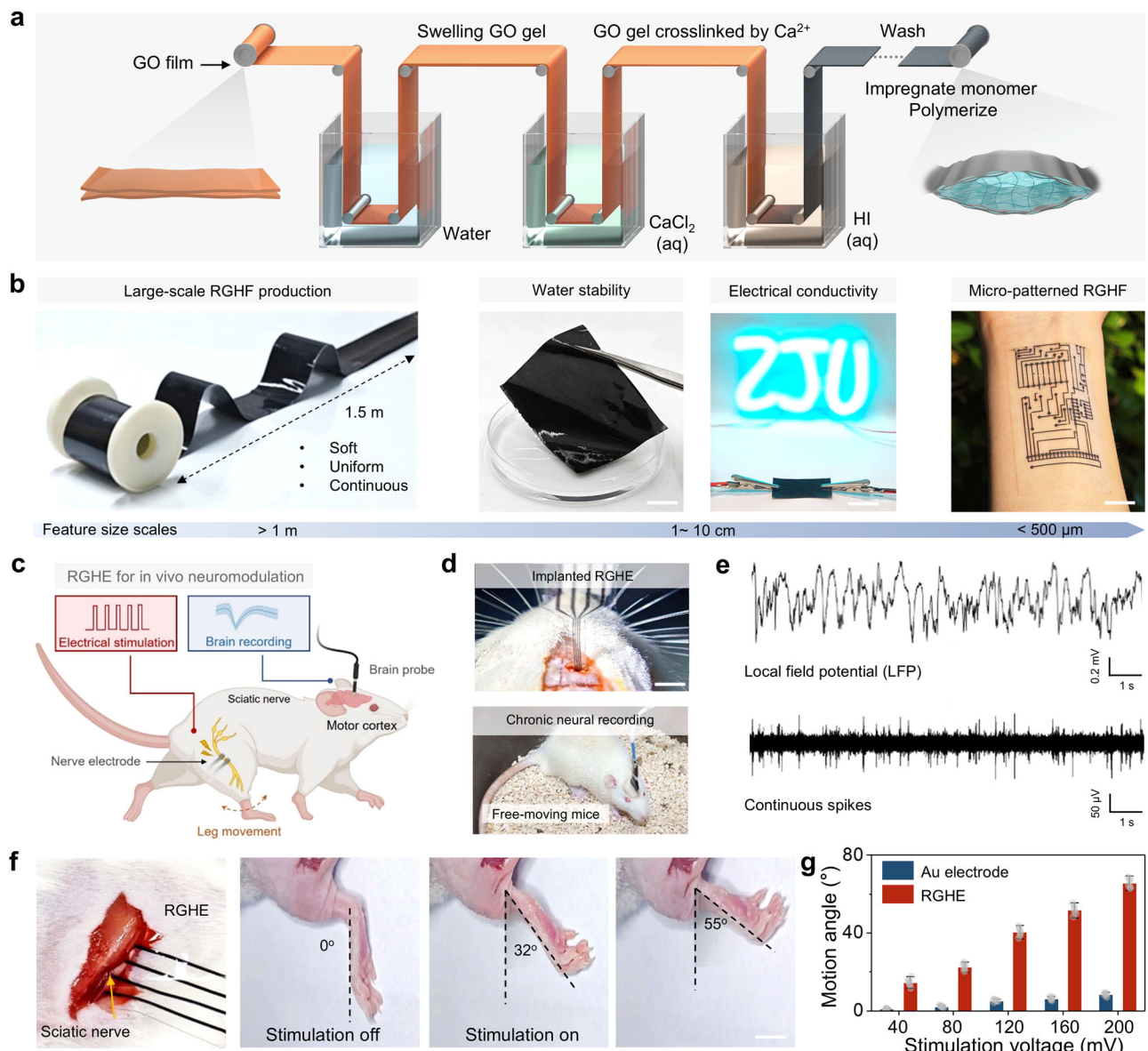


Fig. 6 | Continuous production of RGHEs and their bioelectronic applications.

a The process of scalable production of RGHE. **b** Large-scale and uniform conductive RGHE. Photos exhibit continuity (left), water stability (middle left), electrical conductivity (in conducting circuit for LED, middle right) and the elaborate processed patterns (with minimum feature size of 400 μm , right). **c** Schematic diagram showing the in vivo neuromodulation of the implanted RGHE systems, including electrical stimulation of the sciatic nerve of mouse and neural recording of mouse brain. **d** Photos of a freely behaving mouse with the implanted RGHE for long-term neural recording. Scale bar in the top photo is 1 cm. **e** Representative electrophysiological recordings in the brain by the implanted RGHE. Local field

potential (LFP) traces (0.5 to 250 Hz) under freely moving conditions (Top). Continuous extracellular action potential (AP) traces (300 to 40 kHz) recorded under freely moving conditions (Low). **f** Representative images of in vivo electrical stimulation of sciatic nerve of mouse. Photos of changes in the joint angle of a leg (difference between the maximum and minimum ankle joint movement degree) on sciatic nerve stimulation (200 mV, 1 Hz) using the RGHE. Scale bar is 1 cm. **g** The changes in the joint angle in response to stimulation voltage (40–200 mV, 1 Hz) using RGHE and the commercial Au electrode. The data was expressed as mean $\pm$ SD with a sample size of 6.

Preparation of GO with different C/O

GOs with different C/O ratios were prepared by ultraviolet (UV) reduction. GOs with C/O of 1.79, 1.90, 1.94 were prepared by exposure to UV for 1, 3, 6 h (Supplementary Fig. 18a). C/O ratio was measured by X-ray photoelectron spectroscopy (XPS, Supplementary Fig. 6).

Cytotoxicity and biocompatibility

The cell cytotoxicity of RGHE was measured with NIH3T3 cells using the CCK-8 assay kit. The sterilized hydrogel disks (8 mm in diameter) were placed into a clear 24-well cell plate. NIH3T3 cells were seeded on the hydrogel disks at a density of 4.0×10^4 cells per well, and incubated in 5% CO_2 humidified atmosphere at 37 °C. According to the above

grouping treatment, incubation for 1 day, 3 days, and 5 days. The culture medium was removed by washing each well three times with phosphate-buffered saline. 1 mL of 10% CCK-8 was added into each well, followed by incubation for another 2 h at 37 °C. The optical density was then measured using a Cytation 1 Cell Imaging Multi-Mode Reader (BioTek Instrument, USA) at 450 nm. Growth medium without sample was used as control.

In vivo neural signal recording

After a 7-day acclimatization period, mice were anesthetized by isoflurane (1% isoflurane in oxygen). A heating pad at 37 °C was placed underneath the body. Preoperative procedures included shaving the

surgical site on the head followed by aseptic preparation using sequential ethanol and povidone-iodine scrubs. All surgical instruments and implantable RGHE were UV-sterilized for 2 h. The depth of anaesthesia was monitored by pinching the feet of the mice periodically. The mice were fixed in a stereotaxic frame. A craniotomy was made in the skull above the somatosensory cortex region for the implantation of the electrode. Four burr holes were made using a mini drill to anchor the screw into the skull as grounding screws and bone anchors. The RGHE was slowly implanted in the target brain area (AP: +3.4, ML: +3.8; DV: +1.15) using a stereotactic instrument. Following implantation, the devices and incision were secured to the skull surface with non-cytotoxic dental cement, and the surrounding scalp was coated with antibiotic ointment. In vivo neural signal recording was carried out by using the soft RGHE coupling with a 64-channel multi-electrode recording system (Plexon). After the probe implantation, mice were recovered for at least 7 days. Neuronal signals were referenced to two connected skull screws (above the prefrontal cortex and cerebellum). Spike sorting was carried out in Offline Sorter software (Plexon). A 50 Hz notch filter was applied to suppress power line interference. Neural signals were acquired at a sampling rate of 30 kHz. The LFP signals were filtered with a 0.5–250 Hz bandpass, while spike activity was bandpass filtered from 250 Hz to 7.5 kHz. Single-unit activity was identified by setting a detection threshold at three times the standard deviation of the mean signal amplitude. Data from six implanted mice of RGHE and Au electrode (one probe per animal) were analyzed.

In vivo sciatic nerve electrical stimulation

Following a 7-day acclimatization period in the facility, the mice were anesthetized by isoflurane (1% isoflurane in oxygen). A heating pad at 37 °C was placed underneath the body. Prior to surgery, the surgical site on the posterior thigh was shaved and aseptically prepared using sequential alcohol/iodine scrubs. All surgical instruments and implantable devices were UV-sterilized for 2 h. The depth of anaesthesia was monitored by pinching the feet of the mice periodically. A 3-cm skin incision was made on the thigh. The sciatic nerve was exposed by dissecting the vastus lateralis muscle and biceps femoris muscle. The RGHEs or rigid Au electrodes were interfaced with external circuitry through silver wire connections secured with conductive silver paste, then circumferentially positioned around the sciatic nerve with gentle tensioning to ensure stable contact. For neuromuscular activation testing, the sciatic nerve was stimulated with biphasic charge-balanced rectangular voltage pulses (1 Hz frequency, 40–200 mV amplitude) using an isolated stimulator (PCSGU250, Velleman). The evoked ankle joint movement was recorded using a digital microscope. The angle of the ankle joint was measured using a protractor marker placed under the leg. After stimulation, the muscles and skin were sutured with surgical sutures. Mice received postoperative monitoring for 2 hours in a thermoregulated recovery chamber before returning to standard housing. Data from six implanted mice of RGHE and Au electrode ($n = 6$, one probe per animal) were analyzed.

Animal experiment protocols

Male C57BL/6J mice (~20–25 g in body weight, 8–10 weeks old) were obtained from the Animal Experimental Center of Zhejiang University (ZJU). Mice were group-housed at a controlled environment with standardized conditions: temperature maintained at ~25 °C, relative humidity at ~60%, and a 12-hour light/dark schedule with food and water ad libitum. The mice were randomly allocated into experimental groups. All animal procedures were compliant with the standard guidelines for the Animal Care and Use of Laboratory approved by the ZJU Ethics Committee, and all animal surgeries were reviewed and approved by the Animal Advisory Committee of ZJU (ZJU20230333).

Immuno-compatibility experiment protocol

For procedures in mice, the RGHE and Au electrodes were firstly cut into discs shape (4 mm in diameter) using a biophysical punch. After sterilization under UV light for 30 min, the samples were implanted subcutaneously into C57BL/6 male mice. The implantation procedure was as follows. In brief, mice were anaesthetized with 3% isoflurane in oxygen, shaved, and the skin was disinfected with iodine. An approximately 8 mm longitudinal incision was made on the dorsal surface, and subcutaneous pockets were created using blunt forceps, about 0.5 cm away from the incision for sample placement. After implantation, the incisions were closed using 5-0 taper-tipped PGA absorbable sutures. The mice were monitored until fully recovered from anesthesia and were raised for 4 weeks post-operation. The mice exhibited normal growth, showed no signs of distress, and maintained stable body weights throughout the entire experimental period.

Retrieval of tissues and samples

At the experimental endpoint (4 weeks post-implantation), mice were sacrificed and the samples along with the surrounding tissues, were excised and collected. All explanted samples were fixed in 10% formaldehyde solution in preparation for histology examination.

Statistics and reproducibility

All experiments were repeated independently for 3 times with similar results without specific annotations, unless noted otherwise. Details of the sample size and appropriate statistical test are included in the figure captions. All data were expressed as mean $\pm$ SD with a sample size of 3, unless noted otherwise. For two-group comparisons, statistical significance was determined using a two tailed unpaired Student's t-test using SPSS Statistics 26.0 or Excel 2024. Statistical significance was set at $p < 0.05$. Statistically significant results are indicated in the figures using * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and no significance (n.s., $p > 0.05$). Error bars represent standard deviation.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All other data supporting the findings of this study are available in the main text and the Supplementary Information. The data generated in this study are provided in the Source Data file. Source data are provided with this paper.

Code availability

All simulation input scripts, data files, and key output datasets generated in this study have been deposited in Figshare and are publicly available at <https://doi.org/10.6084/m9.figshare.30880817>.

References

1. Brodie, B. C. On the atomic weight of graphite. *Philos. Trans. R. Soc. London*. **149**, 249–259 (1859).
2. Gao, W., Alemany, L. B., Ci, L. & Ajayan, P. M. New insights into the structure and reduction of graphite oxide. *Nat. Chem.* **1**, 403–408 (2009).
3. Bagri, A. et al. Structural evolution during the reduction of chemically derived graphene oxide. *Nat. Chem.* **2**, 581–587 (2010).
4. Kim, S. et al. Room-temperature metastability of multilayer graphene oxide films. *Nat. Mater.* **11**, 544–549 (2012).
5. Kumar, P. V. et al. Scalable enhancement of graphene oxide properties by thermally driven phase transformation. *Nat. Chem.* **6**, 151–158 (2014).

6. Wu, J., Lin, H., Moss, D. J., Loh, K. P. & Jia, B. Graphene oxide for photonics, electronics and optoelectronics. *Nat. Rev. Chem.* **7**, 162–183 (2023).
7. Ren, W. & Cheng, H. M. The global growth of graphene. *Nat. Nanotechnol.* **9**, 726–730 (2014).
8. Georgakilas, V. et al. Noncovalent functionalization of graphene and graphene oxide for energy materials, biosensing, catalytic, and biomedical applications. *Chem. Rev.* **116**, 5464–5519 (2016).
9. Yousefi, N., Lu, X., Elimelech, M. & Tufenkji, N. Environmental performance of graphene-based 3D macrostructures. *Nat. Nanotechnol.* **14**, 107–119 (2019).
10. Dreyer, D. R., Park, S., Bielawski, C. W. & Ruoff, R. S. The chemistry of graphene oxide. *Chem. Soc. Rev.* **39**, 228–240 (2010).
11. Erickson, K. et al. Determination of the local chemical structure of graphene oxide and reduced graphene oxide. *Adv. Mater.* **22**, 4467–4472 (2010).
12. Wang, Y. et al. Conformational phase map of two-dimensional macromolecular graphene oxide in solution. *Matter* **3**, 230–245 (2020).
13. Gao, Y. et al. Falling-leaves stacking aggregation of two-dimensional macromolecular graphene oxide in solution. *Langmuir* **39**, 12166–12173 (2023).
14. Yeh, C. N., Raidongia, K., Shao, J., Yang, Q. H. & Huang, J. On the origin of the stability of graphene oxide membranes in water. *Nat. Chem.* **7**, 166–170 (2015).
15. Huang, H., Park, H. & Huang, J. Self-crosslinking of graphene oxide sheets by dehydration. *Chem* **8**, 2432–2441 (2022).
16. Kim, J. et al. Graphene oxide sheets at interfaces. *J. Am. Chem. Soc.* **132**, 8180–8186 (2010).
17. Lodge, T. P. & Ueki, T. Mechanically tunable, readily processable ion gels by self-assembly of block copolymers in ionic liquids. *Acc. Chem. Res.* **49**, 2107–2114 (2016).
18. Gabriel, J. C. P. et al. Swollen liquid-crystalline lamellar phase based on extended solid-like sheets. *Nature* **413**, 504–508 (2001).
19. Ming, X. et al. 2D-topology-seeded graphitization for highly thermally conductive carbon fibers. *Adv. Mater.* **34**, 2201867 (2022).
20. Grimme, S. Do special noncovalent π - π stacking interactions really exist? *Angew. Chem. Int. Ed.* **47**, 3430–3434 (2008).
21. Meyer, J. C. et al. The structure of suspended graphene sheets. *Nature* **446**, 60–63 (2007).
22. Zhang, J. et al. Free folding of suspended graphene sheets by random mechanical stimulation. *Phys. Rev. Lett.* **104**, 166805 (2010).
23. Cao, Y. et al. New structural insights into densely assembled reduced graphene oxide membranes. *Adv. Funct. Mater.* **32**, 2201535 (2022).
24. Li, M., Zeng, F., Chang, H., Xu, B. & Wang, W. Aggregate structure evolution of low-rank coals during pyrolysis by in-situ X-ray diffraction. *Int. J. Coal Geol.* **116**, 262–269 (2013).
25. Eda, G. et al. Blue photoluminescence from chemically derived graphene oxide. *Adv. Mater.* **22**, 505–509 (2010).
26. Chien, C. T. et al. Tunable photoluminescence from graphene oxide. *Angew. Chem. Int. Ed.* **51**, 6662–6666 (2012).
27. Yuan, W. Z. et al. Changing the behavior of chromophores from aggregation-caused quenching to aggregation-induced emission: development of highly efficient light emitters in the solid state. *Adv. Mater.* **22**, 2159–2163 (2010).
28. Birks, J. B. *Photophysics of Aromatic Molecules* (Wiley, 1970).
29. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy* (Springer, 2006).
30. Rafiee, J. et al. Wetting transparency of graphene. *Nat. Mater.* **11**, 217–222 (2012).
31. Li, Z. et al. Effect of airborne contaminants on the wettability of supported graphene and graphite. *Nat. Mater.* **12**, 925–931 (2013).
32. Compton, O. C. et al. Tuning the mechanical properties of graphene oxide paper and its associated polymer nanocomposites by controlling cooperative intersheet hydrogen bonding. *ACS Nano* **6**, 2008–2019 (2012).
33. Qin, Z. & Buehler, M. Bioinspired design of functionalised graphene. *Mol. Simulat.* **38**, 695–703 (2012).
34. Ling, S. et al. Design and function of biomimetic multilayer water purification membranes. *Sci. Adv.* **3**, e1601939 (2017).
35. Liu, L., Zhang, R., Liu, Y., Tan, W. & Zhu, G. Insight into hydrogen bonds and characterization of interlayer spacing of hydrated graphene oxide. *J. Mol. Model.* **24**, 137 (2018).
36. Medhekar, N. V., Ramasubramaniam, A., Ruoff, R. S. & Shenoy, V. B. Hydrogen bond networks in graphene oxide composite paper: structure and mechanical properties. *ACS Nano* **4**, 2300–2306 (2010).
37. Do, K. U., Conner, A. V. & Wheeler, S. E. Making Sense of Heteroatom Effects in π - π Interactions. *J. Am. Chem. Soc.* **147**, 32273–32286 (2025).
38. Zhao, X. et al. Polyelectrolyte-stabilized graphene oxide liquid crystals against salt, pH, and serum. *Langmuir* **30**, 3715–3722 (2014).
39. Ciesielski, A. & Samori, P. Supramolecular approaches to graphene: from self-assembly to molecule-assisted liquid-phase exfoliation. *Adv. Mater.* **28**, 6030–6051 (2016).
40. Flory, P. J. & Rehner, J. Statistical mechanics of cross-linked polymer networks II. Swelling. *J. Chem. Phys.* **11**, 521–526 (1943).
41. Flory, P. J. *Principles of Polymer Chemistry* (Cornell University Press, 1953).
42. Chang, D. et al. Reversible fusion and fission of graphene oxide-based fibers. *Science* **372**, 614–617 (2021).
43. Sun, Z., Fang, S. & Hu, Y. H. 3D graphene materials: from understanding to design and synthesis control. *Chem. Rev.* **120**, 10336–10453 (2020).
44. Yuk, H., Lu, B. & Zhao, X. Hydrogel bioelectronics. *Chem. Soc. Rev.* **48**, 1642–1667 (2019).
45. Wu, J. et al. Adhesive anti-fibrotic interfaces on diverse organs. *Nature* **630**, 360–367 (2024).
46. Won, D. et al. Laser-induced wet stability and adhesion of pure conducting polymer hydrogels. *Nat. Electron.* **7**, 475–486 (2024).
47. Han, I. K. et al. Electroconductive, adhesive, non-swelling, and viscoelastic hydrogels for bioelectronics. *Adv. Mater.* **35**, 2203431 (2023).
48. Li, G. et al. Highly conducting and stretchable double-network hydrogel for soft bioelectronics. *Adv. Mater.* **34**, 2200261 (2022).

Acknowledgements

This work is financially supported by the National Key Research and Development Program of China (2022YFA1205300 and 2022YFA1205301 to Z.X.), National Natural Science Foundation of China (Nos. 52122301 to Z.X., 52090030 to C.G., 52272046 to Y.J.L., 12425201 to Z.P.X. and 52303284 to Y.W.), “Pioneer” and “Leading Goose” R&D Program of Zhejiang (2023C01190 to Z.X.), the Fundamental Research Funds for the Central Universities (226-2024-00074 to Z.X. and 226-2023-00023 to Y.J.L.), International Research Center for X Polymers, International Campus, Zhejiang University (No. 130000-171207723/001/014 to Y.W.) and the fellowship of China National Postdoctoral Program for Innovative Talents (BX20230309 to D.C.). We thank staffs from Testing and Analysis Center of Department of Polymer Science and Engineering at Zhejiang University: Yi Guo for SEM, Yingying Zhang for CLSM and fluorescence spectrophotometer, Xinning Zhang for AFM and TEM.

Author contributions

Conceptualization, Y. Gao, X. Ming, Z. Xu, Z. P. Xu; Methodology, Y. Gao, Y. Wang, Y. C. Liao, X. Ming, R. M. Li, Z. Xu, Z. P. Xu and X. L. Zhao; Investigation, Y. Gao, Y. C. Liao, Y. Wang, X. Ming, R. M. Li, D. Chang, L. Peng, H. Li, K. W. Li and M. Cao; Writing-Original Draft, Y. Gao, Y. C. Liao,

Y. Wang, X. Ming, Z. Xu, Z. P. Xu, Y. J. Liu, C. Gao and R. M. Li; Writing-Review & Editing, Y. Gao, X. Ming, Y. C. Liao, Z. Xu, and Z. P. Xu; Funding Acquisition, Z. Xu and Z. P. Xu; Supervision, Z. Xu, Z. P. Xu and X. Ming. All authors participated in the discussion and comments of the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41467-026-71003-z>.

Correspondence and requests for materials should be addressed to Xin Ming, Zhiping Xu or Zhen Xu.

Peer review information *Nature Communications* thanks In Woo Cheong, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

Reprints and permissions information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026