Supplementary Information S2.

S2A *: Effective diameter:*

Effective diameter was calculated using the following formula:

$$D_{eff} = D_{bare} + \kappa^{-1}(\ln A' + K_E + \ln 2 - 1/2) \quad \text{eq. S1}$$

Where K_E is the Euler's constant (0.5772).

D_{bare}, the collagen bare diameter corresponding to structural diameter without consideration of electrostatic effect, was set at 1.5 nm according to ref. 1.

κ is the Debye screening length calculated as in ref. 2:

$$\kappa^2 = \left[4\pi l_B \left(2n_s + \delta C' \alpha z_p\right)\right] \quad \text{eq. S2}$$

Where l_B is the Bjerrum length (0.714 nm at 25°C).

n_s is the free anions concentration.

δ is the Donnan salt exclusion coefficient.

C' is the polyelectrolyte concentration at which we want to determine the effective diameter.

αz_p is the net charge per polyelectrolyte at a given pH.

A' is related to the charge per unit length. To calculate the net charge, we have used, as in ref. [4, 5], the charge calculation tool made available by Gale Rhodes [http://spdbv.vital-it.ch/TheMolecularLevel/Goodies/PeptChg.xls] and we took into account that 20% of the lysines from rat tendon collagen are hydroxylated [6]. At pH 2.5 and pH 3.5, the collagen net charge Q is +257 and +231 respectively. Since collagen is a weakly charged polyelectrolyte, it allows the use of the Debye-Huckel approximation. Then A' is calculated as follows:

$$A' = 2\pi \left(\nu_{eff}\right)^2 Q g^{-1} \kappa^{-1} \quad \text{eq. S3}$$

ν_{eff}, the effective linear surface density, was calculated according to the condensation concept [3].

$$g = \left(\kappa r_{bare}\right)^2 \left[K_1\left(\kappa r_{bare}\right)\right]^2 e^{2\kappa r_{bare}} \quad \text{eq. S4}$$

Where K_1 is the Bessel function of the first order and r_{bare} is the half of the bare diameter (0.75 nm).

The effective diameter was thus calculated inserting equations S2, S3 and S4 into equation S1.

S2B : *Persistence length:*

The calculation of the persistence length was based on the empirical relationship issued from the work of Chen [7] and Khoklov and Semenov [8]:

$$C_{I/N} = \frac{\left(a_1\alpha + a_2\alpha^2 + a_3\alpha^3\right)}{1 + b_1\alpha + b_2\alpha^2} \qquad \text{eq. S5}$$

Where $C_{I/N}$ is the concentration at the isotropic to nematic transition. $\alpha = L/l_p$, where L is the polymer length (300 nm in the case of collagen I) and l_p its persistence length to be determined. a_1, a_2, a_3, b_1 and b_2 are respectively 26.1020, 66.0026, 117.8460, 3.4806 and 9.0331. $C_{I/N}$ is obtained from *in situ* experimental measurements in the glass-microchambers (See material and method).
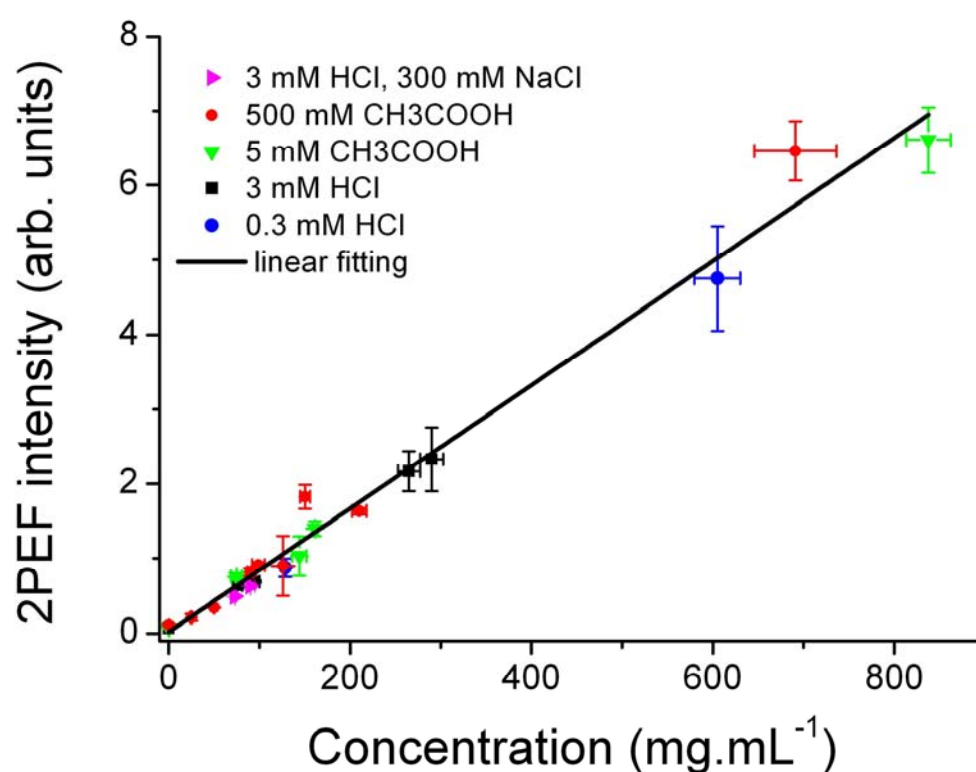

Figure S1: Calibration of the endogenous fluorescence of collagen solutions. Homogeneous collagen solutions with different concentrations were prepared by centrifugation at 14 000 g using tubes of 3 kD filters (VIVASPIN 20, Sartorius). Concentration of those samples were checked for their hydroxyproline amount and used for calibration of the multiphoton setup. These solutions were put between 2 glass coverslips and imaged using the same conditions as

for microchambers. Figure SI-1 displays our 2PEF measurements in these solutions versus concentration. Concentrations are given as average values ± standard deviation calculated from three independent titrations. 2PEF intensities are given as average value ± standard deviation calculated from 5 to 10 images recorded in every sample with a given concentration. Compared analysis of the background noise and of the quite low endogenous 2PEF response from the collagen solutions showed that the collagen 2PEF was significant above 15 mg.mL-1. 2PEF signal shows a linear relationship with the concentration and the data from all physico-chemical conditions fit on the same line. This calibration enabled in situ measurement of the concentration within the microchambers gradient and correlation with the liquid crystal texture revealed by the SHG image.

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