

## **Beta-Adrenoceptor Antagonists Affect Amyloid Nanostructure; Amyloid hydrogels as drug delivery vehicles**

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### **Supplementary Information**

#### **Experimental details**

##### **Drug loaded amyloid hydrogel preparation**

Drug loaded amyloid hydrogels were produced as follows, using a modified method developed by Knowles *et al.* (see Knowles *et al.*, *Nature Nanotechnology*, 2010, **5**, 204-207): Hen egg white Lysozyme (Sigma Aldrich, Dorset, UK) was dissolved in aqueous 10mM hydrochloric acid as a 5% w/v solution and either atenolol, propranolol hydrochloride or timolol tartrate (analytical grade purity, Sigma Aldrich, Dorset, UK) was added to the lysozyme solution at a concentration of 1% w/v. The drug loaded lysozyme solution was incubated at 65 °C until hydrogel formation occurred, resulting in a drug loading of 100 %. Lysozyme amyloid hydrogel formation typically occurs in the order of 14 days at high temperatures (> 50 °C) and low pH, resulting in a clear gel with typical elastic modulus values ( $G'$ ) of below 50 Pa (reflecting the relatively low loading, 5% w/v). It was observed that gelation onset was heavily influenced by drug loading, with gelation onset for atenolol loaded amyloid hydrogels occurring over 3 days, whilst for propranolol loaded it was 7 days and for timolol loaded 14 days. Propranolol and timolol loaded amyloid hydrogels were transparent with lower  $G'$  values. However, atenolol loaded gels were translucent in nature with comparative higher  $G'$  values. Typical  $G'$  values for propranolol and timolol loaded hydrogels were  $\sim 35 \text{ Pa} \pm 8 \text{ Pa}$  but with atenolol loaded hydrogels  $G'$  values were  $\sim 47 \pm 10 \text{ Pa}$  (rheology measurements followed the methodology of Roy *et al.*, *Chemistry-a European Journal*, 2012, **18**, 11723-11731). The differing timescales for the onset of gelation reflect multiple complex physicochemical processes (i.e. drug-lysozyme, drug-amyloid, drug-drug interactions etc.) and is beyond the scope of this communication. However, it does highlight that drug molecules are altering the physicochemical dynamics of protein misfolding.

##### **Drug loaded amyloid hydrogel characterisation**

Atomic force microscopy (AFM): 10  $\mu\text{l}$  of gel sample was deposited onto a freshly cleaved mica surface (G250-2 Mica sheets 1" x 1" x 0.006"; Agar Scientific Ltd, Essex, UK). Each sample was air dried before AFM imaging. The images were obtained by scanning the mica surface in air under ambient conditions using a Bruker MultiMode 8 with a NanoScope V Controller (Bruker UK Ltd, Coventry, UK) operated in scanasyst (peak force) mode. The AFM measurements were obtained using sharp silicon probes (scanasyst-air,  $k \sim 0.4 \text{ N/m}$ , nitride lever ( $l = 600 \text{ nm}$ )) AFM scans were taken at 512 x 512 pixels resolution and produced topographic images of the samples in which the brightness of features increases as a function of height. Typical scanning rates were 1.0 Hz.

Infrared spectroscopy (IR): Fourier transform infrared spectroscopy (IR) was carried out using a Thermo Scientific Nicolet iS10 instrument (Thermo Scientific Ltd, Hemel Hempstead, UK) over the wavenumber range of 575 to 3700  $\text{cm}^{-1}$ . All spectra were corrected for water signals and normalised for comparison. The amide I region of all spectra was peak-fitted using OriginPro 8.5 software and peak FWHM was maintained at a fixed value so as not to over-fit the data.

Circular dichroism (CD): CD spectra of hydrogel samples at a concentration of 1 mg/mL were recorded using an Applied Photophysics Chirascan plus system (Applied Photophysics Ltd, Leatherhead, UK) in a 0.1mm path length circular cell at a bandwidth of 1.0 nm across the wavelength range of 180- 280 nm (scan rate was 15  $\text{nmmin}^{-1}$ , with each spectrum being the accumulation of 6 scans).

### Drug release measurements

Dialysis membrane tubing (cellulose membrane, molecular weight cut-off 12,400; Sigma-Aldrich, Dorset, UK) was prepared by boiling the tubing in distilled water for 30 minutes followed by repeated rinsing with distilled water at room temperature. A fixed weight of 50 mg of drug loaded hydrogel was placed into the tubing and the tubing tightly sealed. The sample was then immersed in a 'sink' volume of ultrapure water (Millipore, 18.2 MΩcm) at 37 °C. Ultrapure water was chosen rather than phosphate buffer solution in order to eliminate specific ion effects influencing structure (see Roy et al. *Chemistry - A European Journal*, 2012, **18**, 11723-11731). Samples of 1 mL were taken from the release medium and replaced with the same amount of fresh medium at given time intervals. Drug concentration within the release medium was determined by ultraviolet (UV) spectroscopy on a Varian Cary50 spectrometer (Agilent Technologies UK Ltd, Workingham, UK) using British Pharmacopoeia 2011  $\lambda_{\text{max}}$  values (Atenolol  $\lambda_{\text{max}}$  = 275 nm, Propranolol  $\lambda_{\text{max}}$  = 292 nm and Timolol  $\lambda_{\text{max}}$  = 297 nm).

### Supplementary figures

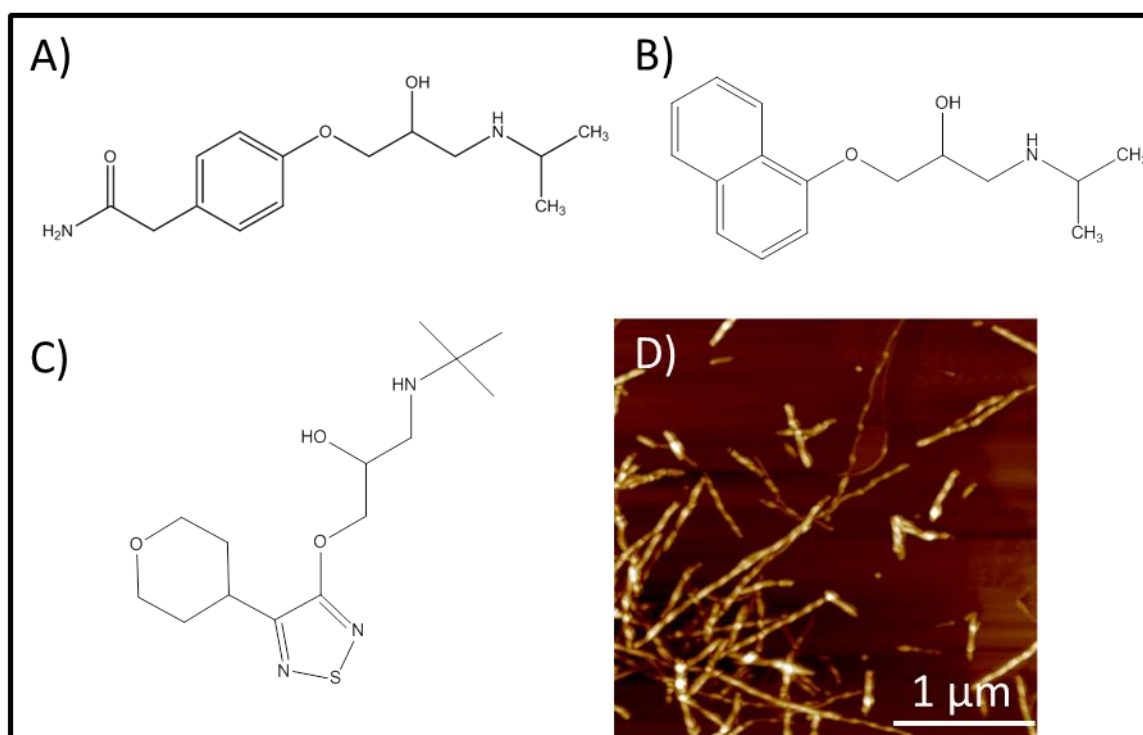


Figure S1. The chemical structures of beta-adrenoceptor antagonists. A) Atenolol; B) Propranolol and c) timolol. D) AFM image of non-loaded amyloid fibres. Varying periodicities can be seen in the fibres.

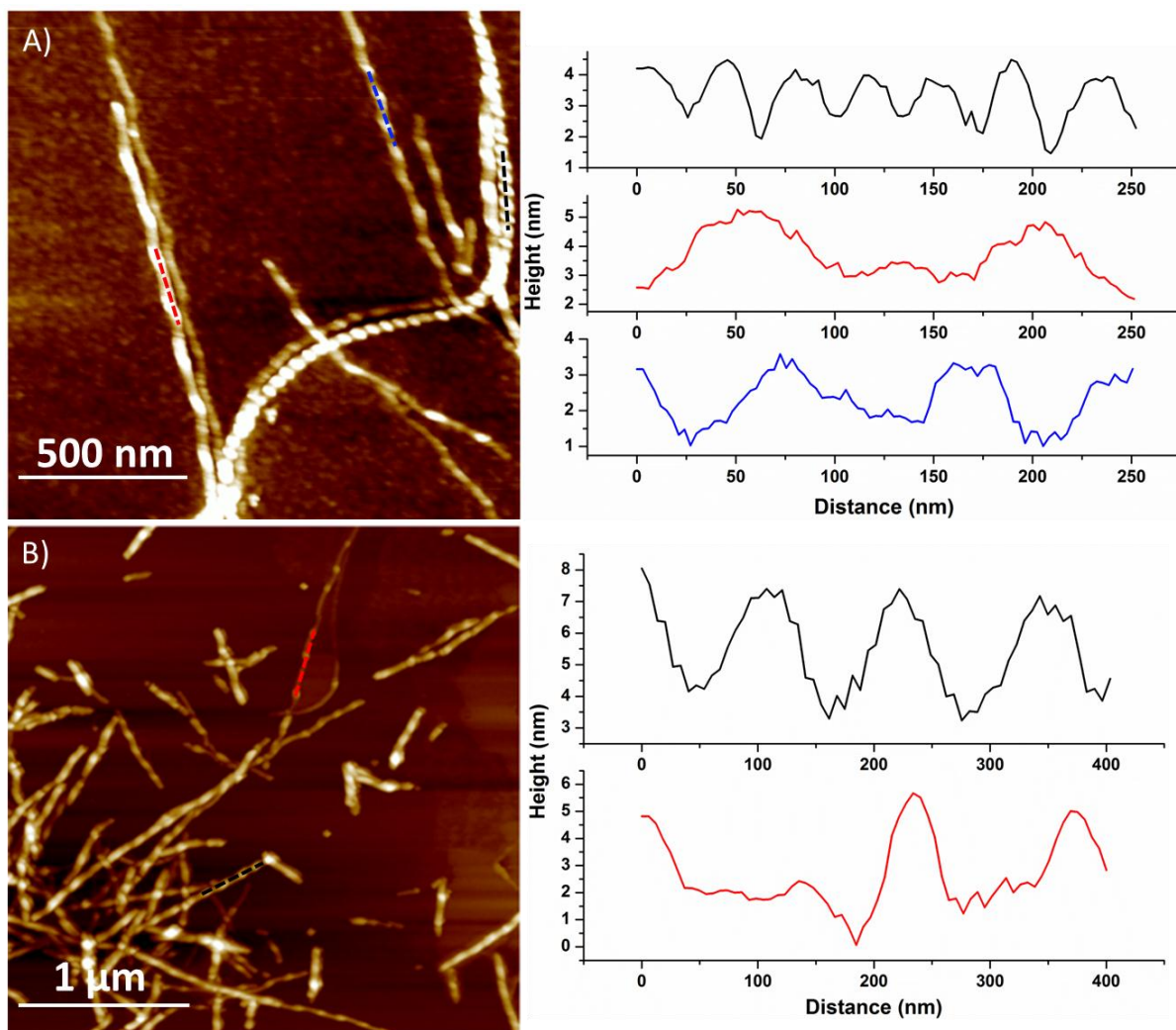


Figure S2. AFM Line profiles. A) Propranolol loaded amyloid fibres. Two fibre periodicities are observed: Short range periodicities (black dashed line and corresponding black line profile (top right)) and long range periodicities (blue and red dashed lines and corresponding blue and red line profiles (top right)). B) Non-loaded amyloid fibres. Multiple fibre periodicities are observed (black and red dashed lines and corresponding black and red line profiles (bottom right) are shown as examples).

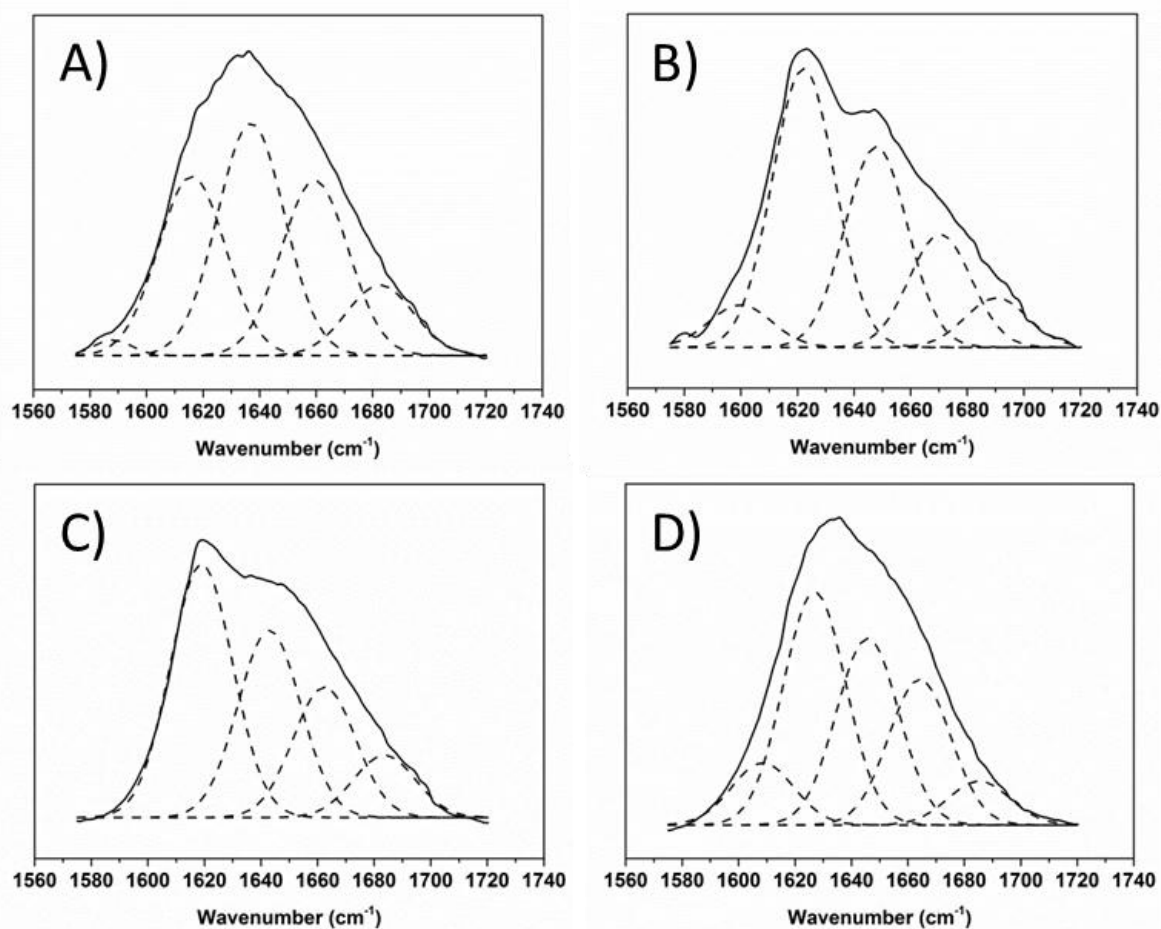


Figure S3. Infrared spectra of the amide I region of drug loaded amyloid fibres. A) Atenolol loaded; B) Propranolol loaded; C) Timolol loaded and D) non-loaded amyloid hydrogel. Peak assignments are as follows: A) 1588  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions), 1616  $\text{cm}^{-1}$  (side-chain moieties/ $\beta$ -sheet contributions), 1637  $\text{cm}^{-1}$  ( $\alpha$ -helix contributions), 1659  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions) and 1682  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions); B) 1600  $\text{cm}^{-1}$  (side-chain moieties/ $\beta$ -sheet contributions), 1623  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions), 1648  $\text{cm}^{-1}$  ( $\alpha$ -helix contributions), 1671  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions) and 1692  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions); C) 1618  $\text{cm}^{-1}$  (side-chain moieties/ $\beta$ -sheet contributions), 1642  $\text{cm}^{-1}$  ( $\alpha$ -helix contributions), 1662  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions) and 1682  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions); D) 1608  $\text{cm}^{-1}$  (side-chain moieties/ $\beta$ -sheet contributions), 1627  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions), 1646  $\text{cm}^{-1}$  ( $\alpha$ -helix contributions), 1665  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions) and 1686  $\text{cm}^{-1}$  ( $\beta$ -sheet contributions). See the following references for peak assignments: P. Roach *et al.*, *Journal of the American Chemical Society*, 2005, **127**, 8168-8173; L. Z. Polzi *et al.*, *Journal of the American Chemical Society*, 2011, **133**, 11414-11417 and P. Juszczak *et al.*, *Journal of Peptide Science*, 2009, **15**, 23-29., S. A. Petty *et al.*, *Journal of the American Chemical Society*, 2005, **127**, 13488-13489 and S. A. Petty *et al.*, *Proceedings of the National Academy of Sciences of the United States of America*, 2005, **102**, 14272-14277.

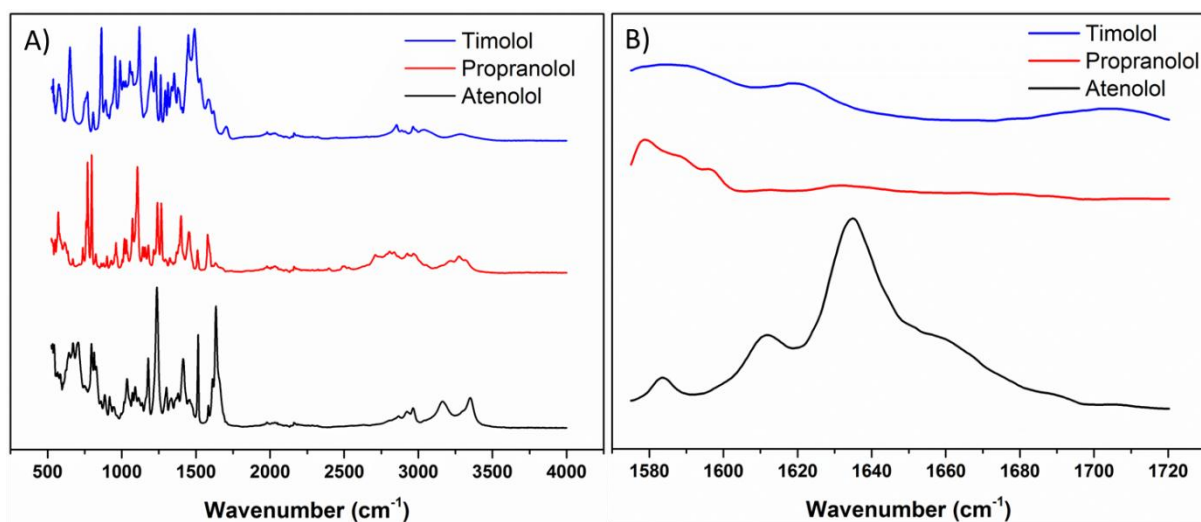


Figure S4. The normalised infrared spectra of the beta-adrenoceptor antagonists. A) Full region scan. B) Amide I region.

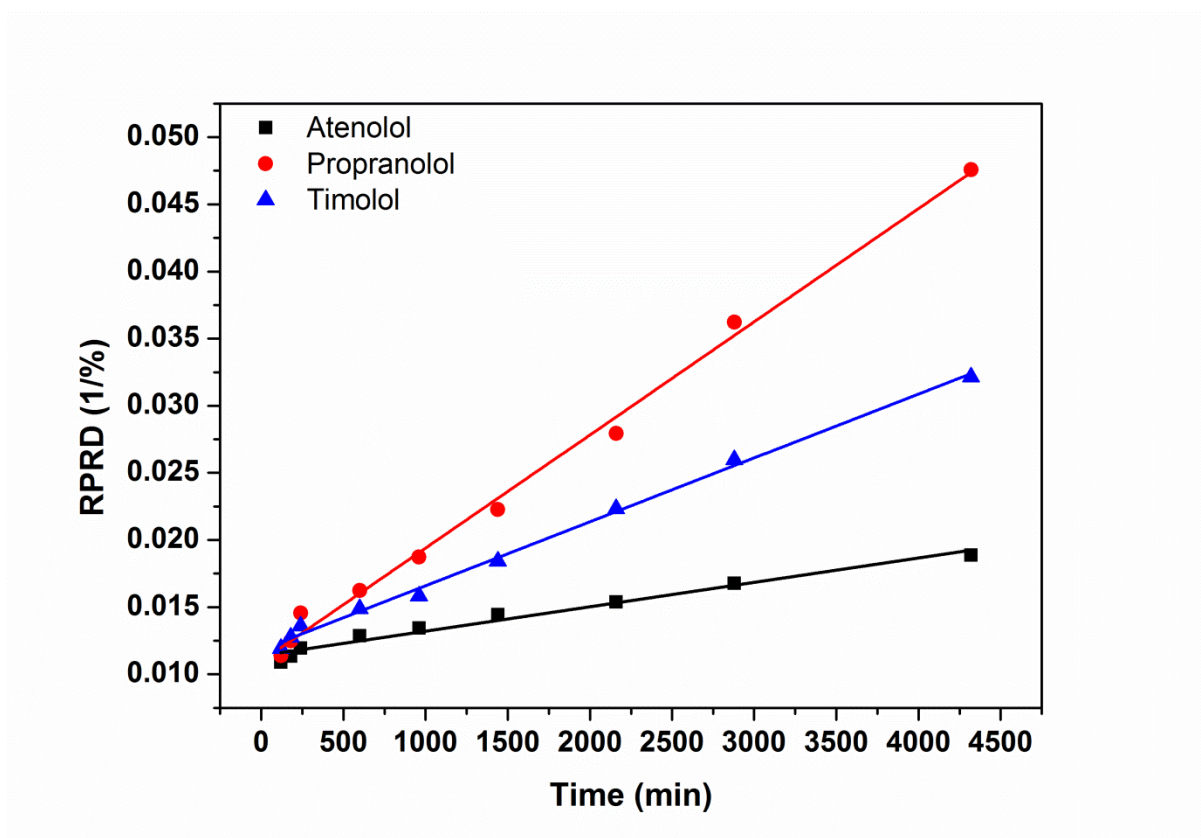


Figure S5. Reciprocal percentage release data against time. Following standard 2<sup>nd</sup> order exponential mathematics (i.e. 1/A vs. t) gives the following straight line R<sup>2</sup> values: 0.97 (atenolol), 0.99 (propranolol) and 0.97 (timolol).