In order to investigate different model of transcriptional regulation, we calculated exact temporal mRNA distributions using Chemical Master Equation (CME) following approach by (Gomez-Schiavon et al., 2017) using fast matrix exponent function in MATLAB (Al-Mohy and Higham, 2011). Genetic algorithm (GA) was implemented using the *ga* function in MATLAB and employed to estimate model parameters (allowing consideration of up to 2000 mRNA molecules), minimising the absolute distance between the theoretical (CME) and measured cumulative distribution functions (CDFs). CDFs were fitted using *fitdist* function (with an Epanechnikov kernel function). Best 50 model fits from independent GA runs were obtained for each condition (using 200 population size, elite count 2, 0.6 crossover factor, tournament selection function) using a parallel computation mode. Gene activation rates were constrained below 0.2 min-1 value, *TNFα* degradation rate was constrained between 0.006 and 0.07 min (half-life between 10 and 115 mins), while *IL1β* degradation rate was constrained between 0.002 and 0.006 min (half-life between 115 and ~350 mins). We assumed two independent alleles per gene with the transcription rate constrained by 30 mRNA min-1 per allele. In agreement, rates as high as 2 to 10 mRNA min-1 were reported for specific genes (Molina et al., 2013; Schwanhausser et al., 2011; Skinner et al., 2016; Suter et al., 2011).

Enclosed codes allow fitting smFISH count distribution using genetic algorithm in MATLAB.

* *Tnf/il1b smFISH.xlsx* include measured smFISH counts across experimental conditions for TNFα/IL1β, respectively
* *checking\_dist\_tnf/il1.m* calculates cdf of the mRNA distribution required for the objective function
* *dist\_fit.m* calculates the objective function for the 2-state model of transcription
* *state3\_IL1.m* calculates the objective function for the 3-state model of transcription
* *buildrateMat.m/buildRateMat3.m* calculates theoretical mRNA distribution for the 2 and 3-state model, respectively
* *ga\_run.m* calls genetic algorithm to minimize the distance between measured and theoretical cdf (i.e. the objective function)