Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Modular Droplet Injector for Sample Conservation Providing New Structural Insight for the Conformational Heterogeneity in the Disease-Associated NQO1 Enzyme

Diandra Doppler[1,2], Mukul Sonker[1,2], Ana Egatz-Gomez[1,2], Alice Grieco[3], Sahba Zaare[2,4], Rebecca Jernigan[1,2], Jose Domingo Meza-Aguilar[2], Mohammad T. Rabbani[1,2], Abhik Manna[1,2], Roberto Alvarez[2,4], Konstantinos Karpos[2,4], Jorvani Cruz Villarreal[1,2], Garrett Nelson[2,4], Jay-How Yang[1,2], Jackson Carrion[1,2], Katherine Morin[1,2], Gihan K. Ketawala[1,2], Angel L. Pey[5], Miguel Angel Ruiz-Fresneda[3,6], Juan Luis Pacheco-Garcia[7], Juan A. Hermoso[3], Reza Nazari[2,4], Raymond Sierra[8], Mark S. Hunter[8], Alexander Batyuk[8], Christopher J. Kupitz[8], Robert E. Sublett[8], Stella Lisova[8], Valerio Mariani[8], Sebastien Boutet[8], Raimund Fromme[1,2], Thomas D. Grant[9], Sabine Botha[2,4], Petra Fromme[1,2], Richard A. Kirian[2,4], Jose Manuel Martin-Garcia[3]*, Alexandra Ros[1,2]*

* Co-corresponding authors

1. School of Molecular Sciences, Arizona State University, Tempe, AZ, 85287-1604, USA
2. Center for Applied Structural Discovery, The Biodesign Institute, Arizona State University, Tempe, AZ, 85287-7401, USA
3. Department of Crystallography and Structural Biology, Institute of Physical Chemistry Blas Cabrera, Spanish National Research Council (CSIC), Serrano 119, 28006, Madrid, Spain
4. Department of Physics, Arizona State University, Tempe, AZ, 85287-1504, USA
5. Departamento de Química Física, Unidad de Excelencia en Química Aplicada a Biomedicina y Medioambiente e Instituto de Biotecnología, Universidad de Granada, Av. Fuentenueva s/n, 18071, Granada, Spain
6. Present address: Departamento de Microbiología, Universidad de Granada, Av. Fuentenueva s/n, 18071 Granada, Spain
7. Departamento de Química Física, Universidad de Granada, Av. Fuentenueva s/n, 18071 Granada, Spain
8. Linac Coherent Light Source (LCLS), SLAC National Accelerator Laboratory, Menlo Park 94025 CA, USA
9. Department of Structural Biology, Jacobs School of Medicine and Biomedical Sciences, SUNY University at Buffalo, 955 Main St, Buffalo, NY, 14203, USA

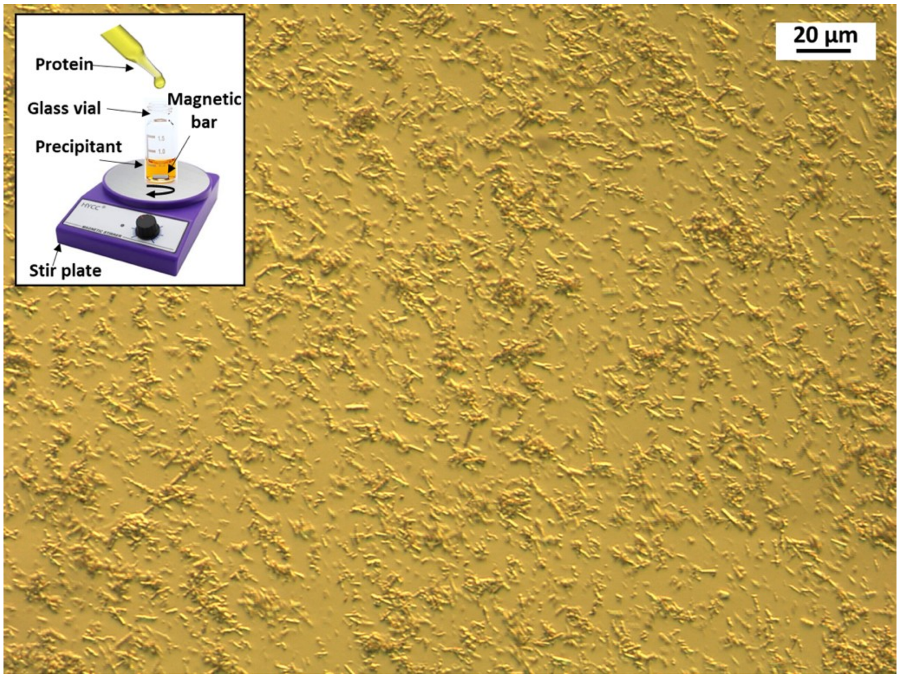

Figure S-1: *Microcrystals of human NQO1. Crystals of 10 x 2 x 2 µm^3 in size were grown by the batch method (inset panel) in 0.1 M Tris pH 8.5, 0.2 M sodium acetate, 20 % PEG 3350 and 20 µM FAD.*

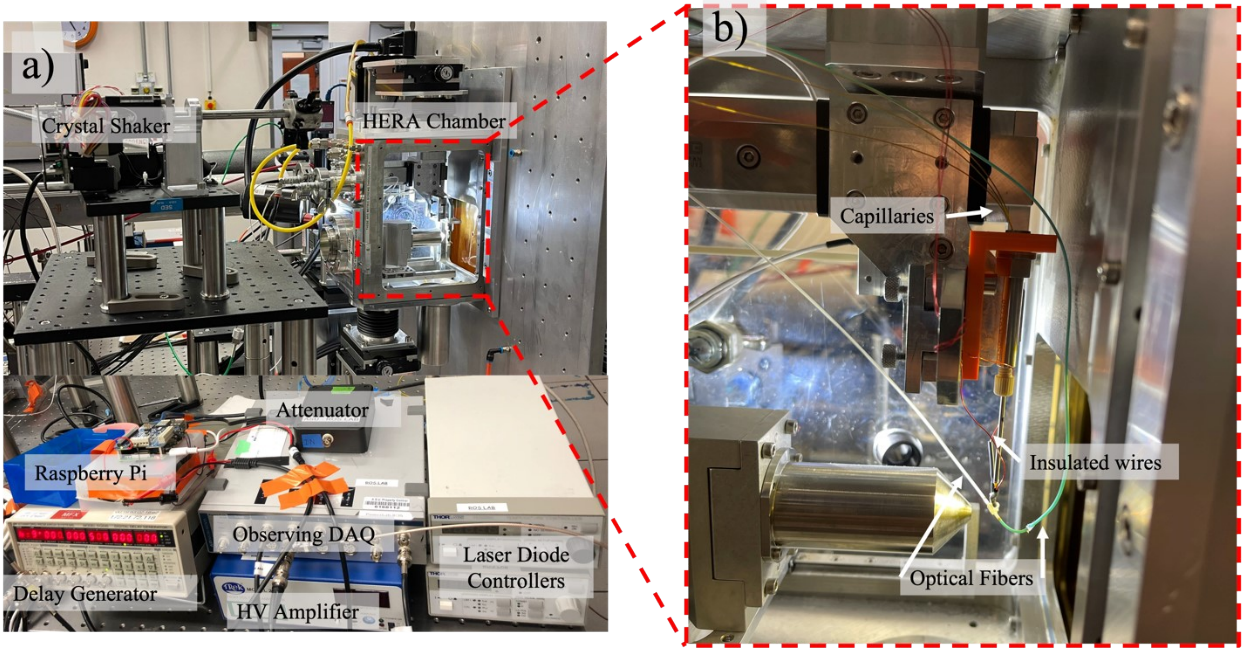

Figure S-2: (a) *Photograph of MFX experimental setup with crystal shaker (left top), HERA chamber (right, top), and other hardware components (bottom) required for droplet generation.* ***(b)*** *Zoomed-in detail of the modular droplet injector installed in the HERA chamber.*

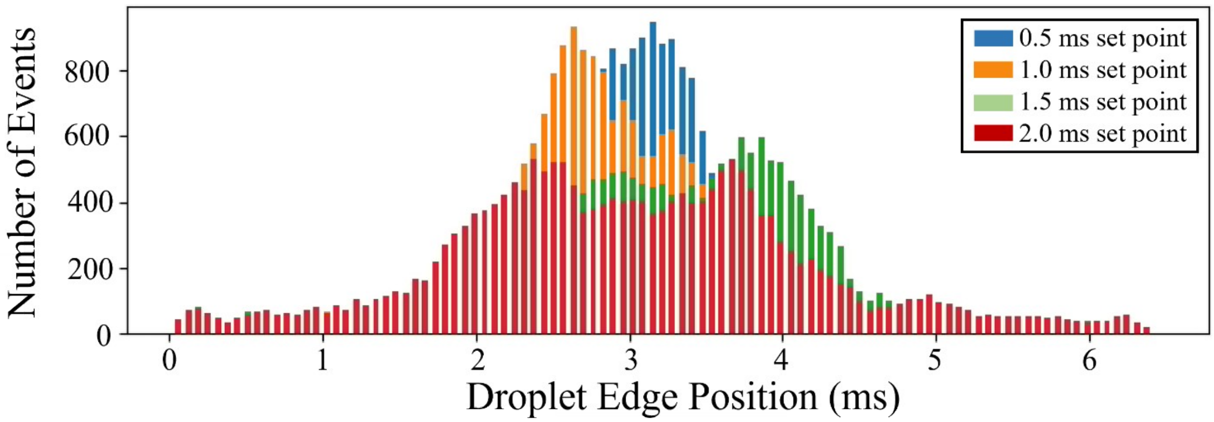

Figure S-3: *Droplet leading edge sweep implementation with NQO1 crystal-laden droplets where leading-edge during a parameter sweep was measured across 0.5, 1.0, 1.5, and 2.0 ms of electrical stimulation durations.*

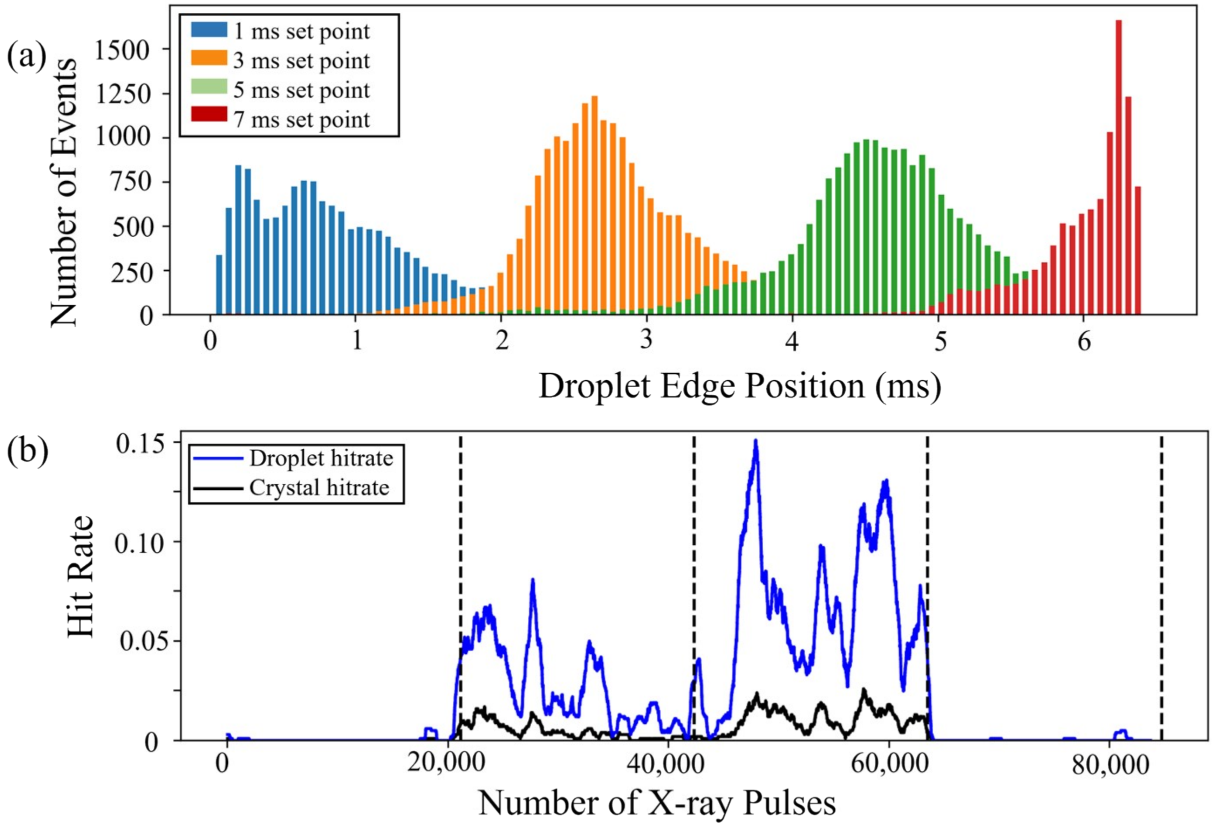

Figure S-4: *Droplet leading edge phase sweep implementation with phycocyanin crystal-laden droplets. (a) Measured leading-edge during a parameter sweep across 1, 3, 5, and 7 ms phase delay set-points. (b) Normalized droplet hit rate (blue) and phycocyanin crystal hit rate (black) during this parameter sweep. Hits for each* ϕ_s *were 5 for 1 ms, 177 for 3ms, 359 for 5 ms and 6 for 7 ms.*

Table S-1: *Comparison of modular droplet injection and continuous injection for three batches of phycocyanin.*

Sample	**Injection Method**	ϕ_s **(ms)**	**Avg Flow (µL/min)**	**Indexed patterns/µL**	**Resolution**
C	Droplets	1, 2, 4, 7	4.5	10.2	1.9 Å
	Droplets	3	5.0	13	
	Droplets	5	4.0	18.2	
D	Droplets	1,2,7	4.4	7.0	1.9 Å
	Droplets	3	4.0	7.8	
	Droplets	5	4.0	2.6	
E	GDVN	-	21.3	5.0	1.9 Å

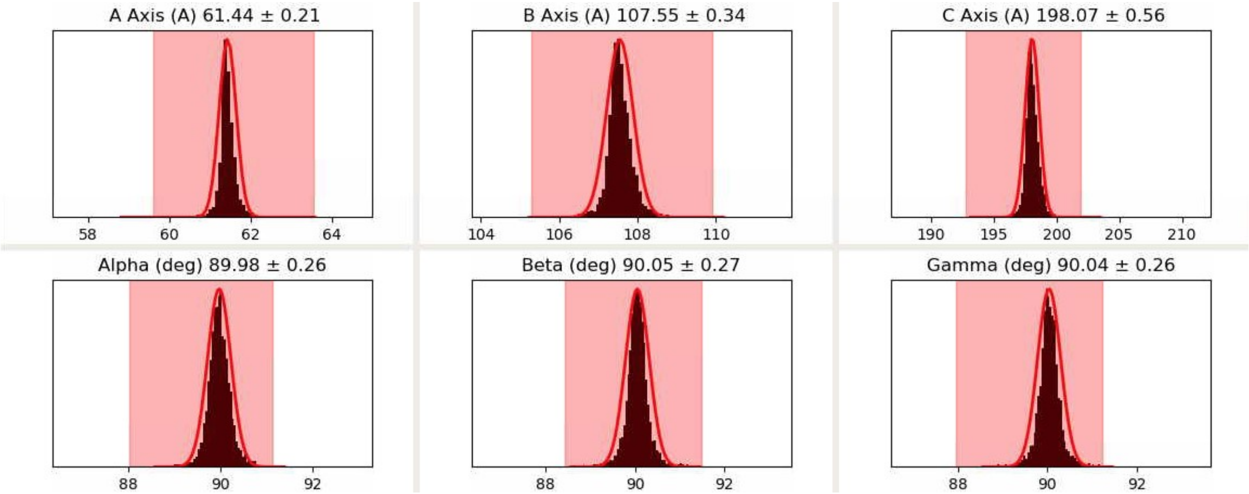

Figure S-5: *Unit cell size distribution. It is shown for the 4446 indexed and integrated diffraction patterns included in the structure solution. A Gaussian function was fit to each distribution, and the mean was calculated using the cell_explorer program from the CrystFEL software package.*

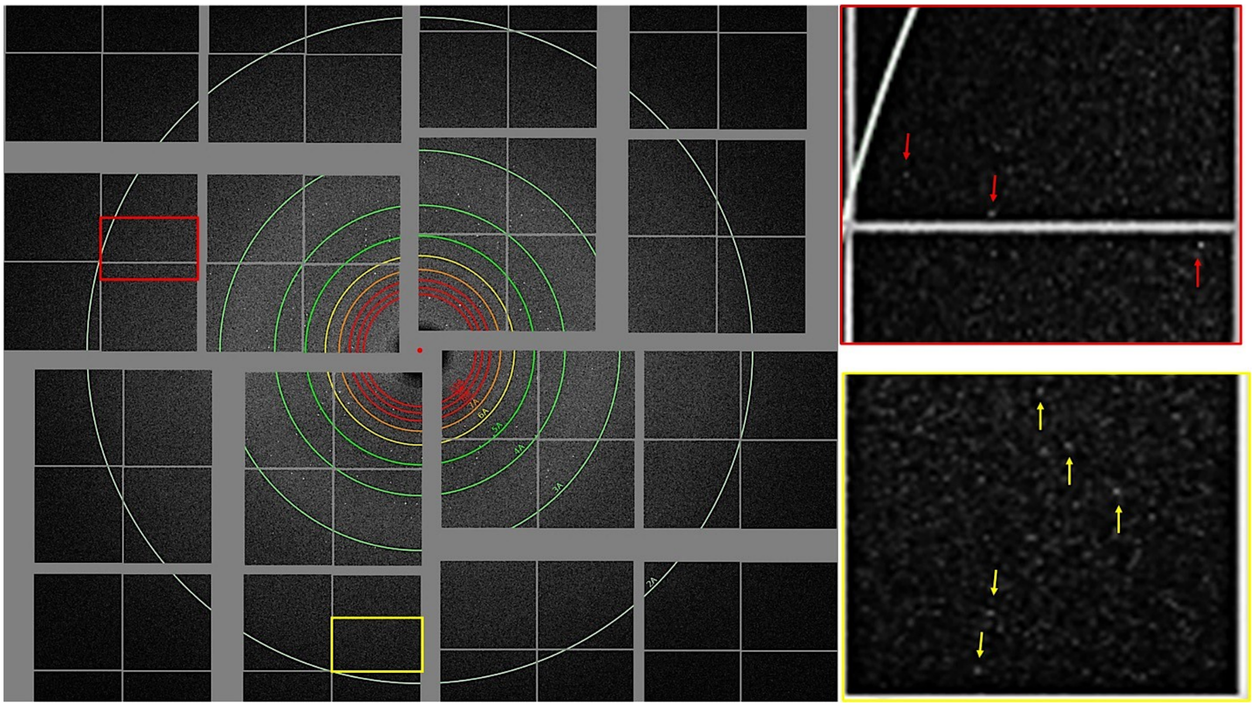

Figure S-6: *Representative diffraction pattern of a single human NQO1 microcrystal. The red and yellow boxes show Bragg reflections up to ~2.2 Å resolution, which have been highlighted by arrows.*

Table S-2. *SFX data collection and refinement statistics.*

Values for the outer shell are given in parentheses.

	NQO1-MDI	Phycocyanin-MDI
Data collection statistics		
XFEL / beamline	MFX / LCLS	MFX / LCLS
Wavelength (Å)	1.285	1.285
Pulse duration (fs)	40	40
Temperature (K)	293	293
Detector	ePix10k	ePix10k
Sample-detector distance (mm)	86.3	86.3

No. of hits / indexed patterns	11364 / 4343	11504 / 7460
Space group	$P2_12_12_1$	H32
a, b, c (Å)	61.4, 107.6, 198.1	188.3, 188.3, 61.1
α, β, γ (°)	90, 90, 90	90, 90, 120
Resolution range (Å)	24.5 – 2.7 (2.8-2.7)	24.6 – 2.0 (2.1-2.0)
No. of unique reflections	36928 (3619)	54596 (5458)
Completeness (%)	100 (100)	100 (100)
Multiplicity	46 (22)	28 (18)
CC*	0.923 (0.520)	0.9140 (0.501)
R_{split}	48.8 (225.6)	49.7 (178.3)
Avg. I/σ (I)	1.9 (0.5)	1.8 (0.6)
Refinement Statistics		
Resolution range (Å)	23.9 – 2.7 (2.8-2.7)	22.3 – 2.0 (2.1-2.0)
No. of reflections, working set	35013	26538
No. of reflections, test set	1822	1380
R_{work}/R_{free}	22.7 / 26.8	20.4 / 25.5
No. of non-H atoms	9118	2763
Protein	8739	2488
Water	163	126
Others	0	1
Ligand	216	147
R.m.s. deviations		

Bond length (Å)	0.005	0.0067
Bond angles (°)	1.162	1.372
Average *B* factors (Å^2)	46.0	26.3
Ramachandran plot		
Favored (%)	97.0	97.9
Allowed (%)	3.0	1.8
Outliers (%)	0	0.3
PDB code	8C9J	8FWA