

## Supplementary information

### **Determinants of neuroglobin plasticity highlighted by joint coarse-grained simulations and high pressure crystallography**

Nathalie Colloc'h<sup>1,\*</sup>, Sophie Sacquin-Mora<sup>2</sup>, Giovanna Avella<sup>3,†</sup>, Anne-Claire Dhaussy<sup>4</sup>, Thierry Prangé<sup>5</sup>, Beatrice Vallone<sup>3</sup>, Eric Girard<sup>6,\*</sup>

<sup>1</sup>ISTCT CNRS UNICAEN CEA Normandie Univ., CERVOxy team, centre Cyceron, 14000 Caen, France

<sup>2</sup>Laboratoire de Biochimie Théorique, CNRS UPR9080, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

<sup>3</sup>Instituto Pasteur–Fondazione Cenci Bolognetti and Dipartimento di Scienze Biochimiche ‘A. Rossi Fanelli’, Sapienza Università di Roma, 5 piazzale Aldo Moro, 00185 Roma, Italy.

<sup>4</sup>CRISTMAT UMR 6508 CNRS ENSICAEN UNICAEN Normandie Univ., 6 bd du Maréchal Juin, 14050 Caen, France.

<sup>5</sup>LCRB, UMR 8015 CNRS Université Paris Descartes, 4 avenue de l’Observatoire, 75270 Paris, France.

<sup>6</sup>Institut de Biologie Structurale (IBS), Université Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France.

<sup>†</sup> Present address: BIOGEM Research Institute.

\*Correspondence should be addressed to N.C. (colloch@cyceron.fr) and E.G. (eric.girard@ibs.fr).

**Supplementary Table 1.** Data collection and refinement statistics.

| Ngb                                   | WT AP        | WT HP-270    | WT HP-310    | V101F AP     | V101F HP-150 | V101F HP-240 | F106W AP     | F106W HP-280 | F106W HP-310 |
|---------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Pressure (MPa)                        | 0.1          | 270          | 310          | 0.1          | 150          | 240          | 0.1          | 280          | 310          |
| Resolution (Å)                        | 2.00         | 1.90         | 2.05         | 2.00         | 1.95         | 2.40         | 1.75         | 2.15         | 2.10         |
| Cell                                  |              |              |              |              |              |              |              |              |              |
| a (Å)                                 | 88.32        | 87.56        | 87.37        | 89.02        | 87.93        | 87.2         | 88.78        | 86.21        | 85.99        |
| c (Å)                                 | 114.46       | 113.64       | 113.28       | 113.88       | 113.7        | 113.9        | 114.31       | 114.46       | 114.58       |
| Cell volume (Å <sup>3</sup> )         | 773219       | 754525       | 748874       | 781544       | 761317       | 750455       | 780270       | 736715       | 733728       |
| Completeness (%)                      | 98.7 (99.7)  | 99.8 (99.7)  | 97.6 (97.8)  | 97.7(97.9)   | 99.4 (99.7)  | 100 (100)    | 99.8 (99.8)  | 100 (100)    | 99.5 (99.9)  |
| Multiplicity                          | 4 (4.1)      | 4.7 (4.7)    | 5 (5)        | 5.3 (5.4)    | 4.1 (4.2)    | 5 (5.1)      | 4.5 (4.6)    | 8.8 (8.9)    | 4.2 (4.2)    |
| Unique reflections                    | 11623 (1685) | 13380 (1908) | 10340 (1509) | 11681 (1669) | 12433 (1807) | 6714 (966)   | 17681 (2551) | 9151 (1318)  | 9673 (1396)  |
| R merge (%)                           | 10.4 (47.9)  | 9.3 (47.3)   | 11.2 (54.1)  | 12.9 (87.2)  | 8.9 (72.9)   | 18.3 (78.1)  | 7.8 (68.8)   | 15.1 (86.3)  | 12.6 (86.6)  |
| Mean I/σ(I)                           | 10.2 (3.3)   | 10.3 (3.3)   | 9.8 (3.2)    | 9.4 (2)      | 10.2 (1.8)   | 4.9 (1.8)    | 10.8 (1.7)   | 10.2 (3.3)   | 7.4 (1.6)    |
| Rwork (%)                             | 14.31        | 16.86        | 17.15        | 15.52        | 15.29        | 14.37        | 15.38        | 17.06        | 16.45        |
| Rfree (%)                             | 18.05        | 22.14        | 23.71        | 20.9         | 20.27        | 26.03        | 18.65        | 23.8         | 26.02        |
| Mean standard deviation from ideality |              |              |              |              |              |              |              |              |              |
| Length (Å)                            | 0.019        | 0.02         | 0.023        | 0.021        | 0.02         | 0.033        | 0.015        | 0.027        | 0.028        |
| Angle (°)                             | 1.879        | 2.01         | 2.219        | 1.972        | 2            | 2.922        | 1.685        | 2.451        | 2.602        |
| Mean B factors (Å <sup>2</sup> )      |              |              |              |              |              |              |              |              |              |
| Protein                               | 30.4         | 32.1         | 33.7         | 28.9         | 31.9         | 39.3         | 30.9         | 42.1         | 41.1         |
| Heme                                  | 19.3         | 21.9         | 21.3         | 21.8         | 21.7         | 26           | 19.3         | 20.4         | 21           |
| Water                                 | 38.3         | 37.7         | 35.6         | 40.3         | 41           | 35.8         | 42.6         | 40.2         | 41.9         |
| Number water                          | 76           | 81           | 71           | 76           | 76           | 51           | 98           | 73           | 85           |
| PDB code                              | 5eet         | 5eoh         | 5eqm         | 5eu2         | 5ev5         | 5ey5         | 5eus         | 5f0b         | 5f2a         |

Data between parentheses correspond to the highest resolution shell.  $*R_{\text{sym}} = \frac{\sum_{h,k,l} \sum_i |I_i(h,k,l) - \langle I_i(h,k,l) \rangle|}{\sum_{h,k,l} \sum_i I_i(h,k,l)}$ , where  $I_i(h,k,l)$  is the intensity of observed reflections and  $\langle I_i(h,k,l) \rangle$  the weighted mean of all observations after rejection of outliers.  $\$R_{\text{work}} = \frac{\sum |F_o| - |F_c|}{\sum |F_o|}$ ; indicates accuracy of the model.  $\#R_{\text{free}}$  is a cross validation residual using 5% of the native data which were randomly chosen and excluded from the refinement.

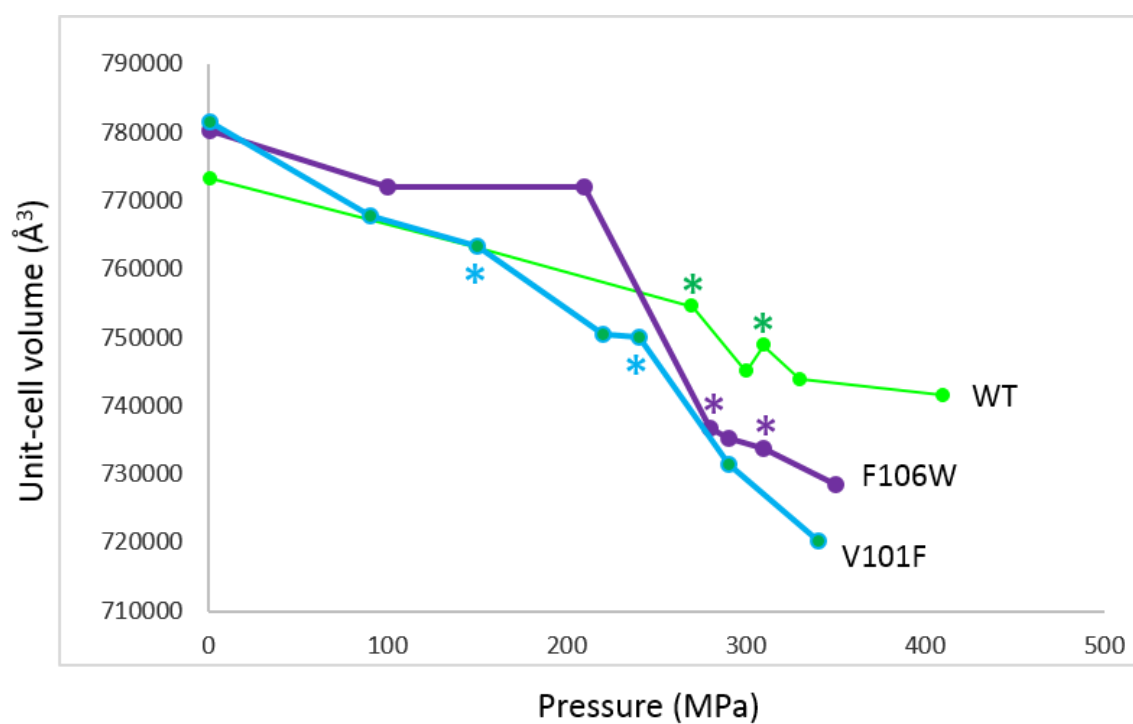
**Supplementary Table 2.** Internal cavity volumes (in Å<sup>3</sup>)

| WT<br>AP | WT<br>HP-270 | WT<br>HP-310 | V101F<br>AP | V101F<br>HP-150 | V101F<br>HP-240 | F106W<br>AP | F106W<br>HP-280 | F106W<br>HP-310 |
|----------|--------------|--------------|-------------|-----------------|-----------------|-------------|-----------------|-----------------|
| 911      | 829          | 790          | 956         | 944             | 713             | 869         | 830             | 800             |

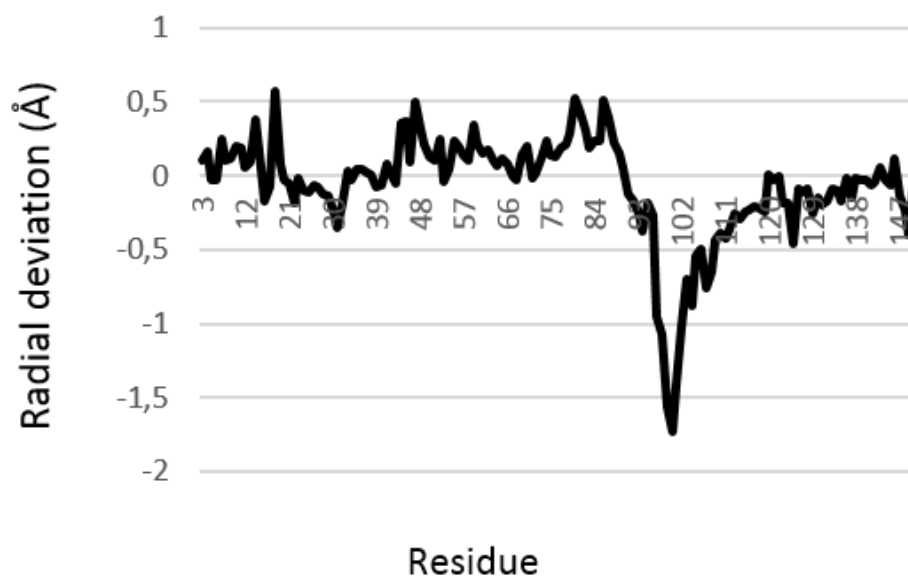
**Supplementary Table 3.** Root mean squares deviation (in Å) calculated on the C $\alpha$  chain between different Ngb structures.

|          | 1W92         | V101F AP     | F106W AP* | WT HP-270 | WT HP-310 |
|----------|--------------|--------------|-----------|-----------|-----------|
| WT AP    | 0.43         | 0.17         | 0.17      | 0.24      | 0.41      |
|          | V101F HP-150 | V101F HP-240 |           |           |           |
| V101F AP | 0.06         | 0.27         |           |           |           |
|          | F106W HP-280 | F106W-HP-310 |           |           |           |
| F106W AP | 0.42         | 0.45         |           |           |           |

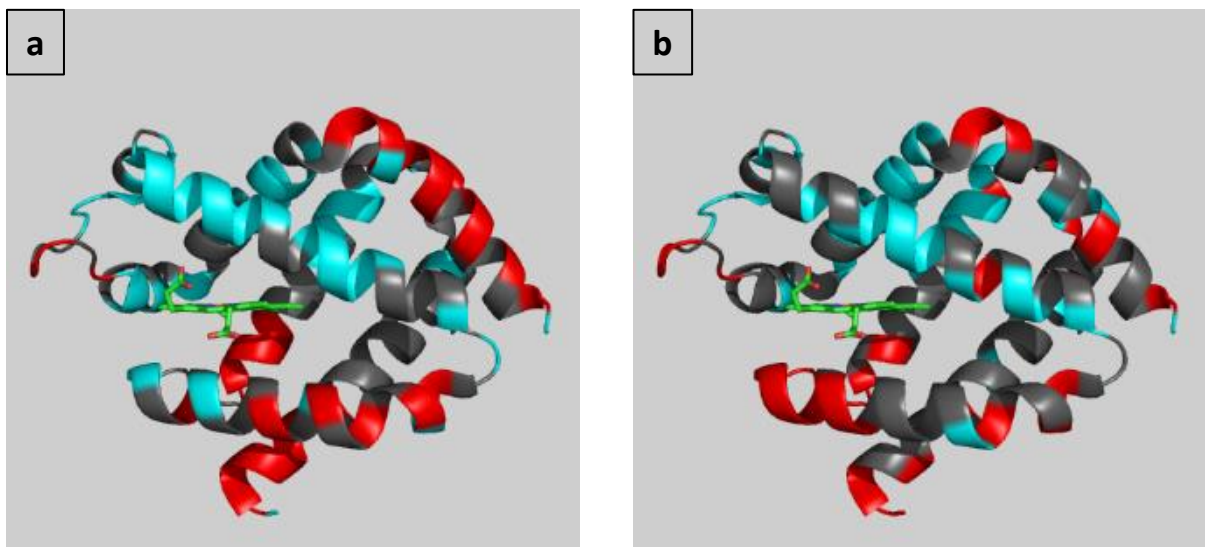
\*Without the C-ter Gly residue



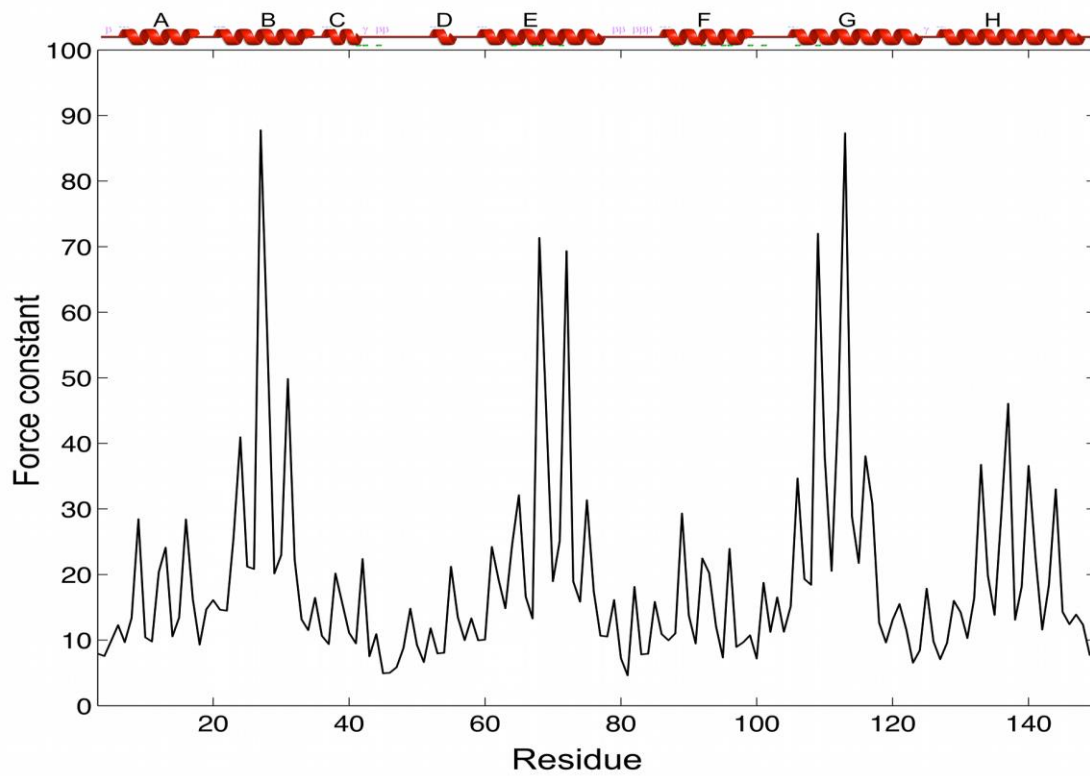
**Supplementary Figure 1.** Unit-cell compressibility curves for NgB WT (in green), V101F (in cyan) and F106W (in purple). The pressure corresponding to the different collected data sets are indicated by stars.



**Supplementary Figure 2.** Radial deviation between Ngb WT HP-310 and AP structures.



**Supplementary Figure 3.** B-factor differences between Ngb structures. Ngb shown in cartoon representation with the heme shown in stick representation. **A.** B-factor differences between WT HP-310 and WT HP-270 (on average  $1.6 \text{ \AA}^2$ ). Residues are colored according to the B-factor differences, in red if B-factor differences are higher than  $4 \text{ \AA}^2$ , in cyan if B-factor differences have negative values, in grey otherwise. **B.** B-factor differences between V101F HP-240 and V101F AP (on average  $10 \text{ \AA}^2$ ). Residues are colored according to the B-factor differences, in red if B-factor differences are higher than  $15 \text{ \AA}^2$ , in cyan if B-factor differences are lower than  $5 \text{ \AA}^2$ , in grey otherwise.



**Supplementary Figure 4.** Ngb WT mechanical profile at ambient pressure.