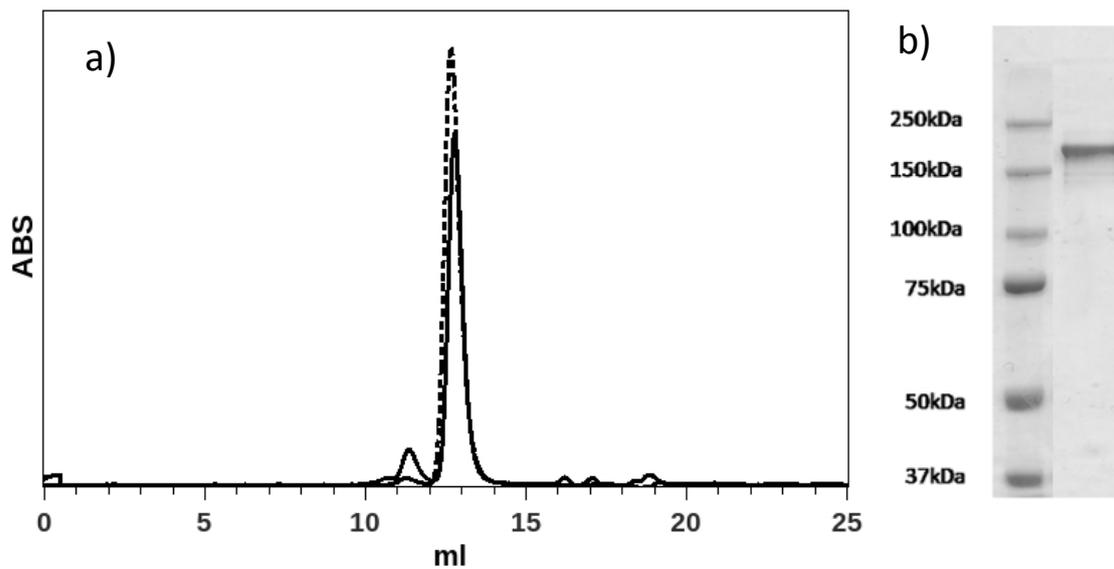


## Supplementary material

### Analysis of anti-CD20 crystals

Anti-CD20 crystals have been dissolved and analyzed by Size Exclusion Chromatography (Figure S1a). The SDS-PAGE electrophoresis performed on the main peak fraction (Figure S1b) shows a single band at MW that is slightly higher than the expected one (it is at 150-160kDa instead of 144kDa). This behavior is not unusual for antibodies and it has been already observed for anti-CD20 protein [1]. MS/MS mode spectroscopy performed on such band fully confirms the presence of intact anti-CD20. Peptide mass fingerprint of each band has been checked against Mascot database by using two modes: Peptide Mass Fingerprint search, which exploits peptide mass values as input, and Sequence Query search, which combines peptide mass data, amino acid sequence, and composition information. Mass spectrometry analysis shows that all bands are compatible with anti-CD20 (IgG1 has a very high sequence homology with anti-CD20) (Table S1).



**Figure S1** Size Exclusion Chromatography for anti-CD20 from washed and dissolved crystals (solid line) and from pristine solution (dashed line) (a). SDS-PAGE electrophoresis performed on-washed and dissolved crystals (b).

MW Band	Gene Info number	Description
> 190 kDa	121039	IgG1 H and Lchain
~ 190 kDa	146387638	Chain L anti-CD20
~ 140 kDa	101688435	IgG1 H chain
75 < MW 100 kDa	146387638	Chain L anti-CD20

**Table S1** Mascot analysis of peptide fingerprint of digested band from anti-CD20 crystals.

Only the sequence showing the best match with experimental data is shown.

## Reference

[1] Isoda Y, Yagi H, Satoh T, Shibata-Koyama M, Masuda K, Satoh M, Koichi K, Shigeru I Importance of the Side Chain at Position 296 of Antibody Fc in Interactions with FcγRIIIa and Other Fcγ Receptors. PLoS ONE, 2015, e0140120

## Macromolecules versus small-molecules crystal diffraction

Powder diffraction is usually exploited for small molecule structure determination and only recently has been applied also to protein molecules [1]. Although many steps forward have allowed the use of this technique also for macromolecules, structural determination still remain very hard to perform due to the strong reflection overlap in the diffraction pattern of such molecules. However, diffraction pattern still represents a precious source of information about the species that are present in the crystal. Indeed, unit cell of small-molecules crystals have volume by far smaller than that of macromolecules crystals due to smaller size of the hosted molecule. Unit-cell dimensions are directly related to properties of reciprocal space lattice, which is the space where diffraction occurs; therefore, small-molecule and macromolecule crystals can be distinguished based on specific features of the diffraction image/pattern. One of the main differences is related to the resolution range in which diffraction occurs. Indeed, according to the Bragg's law

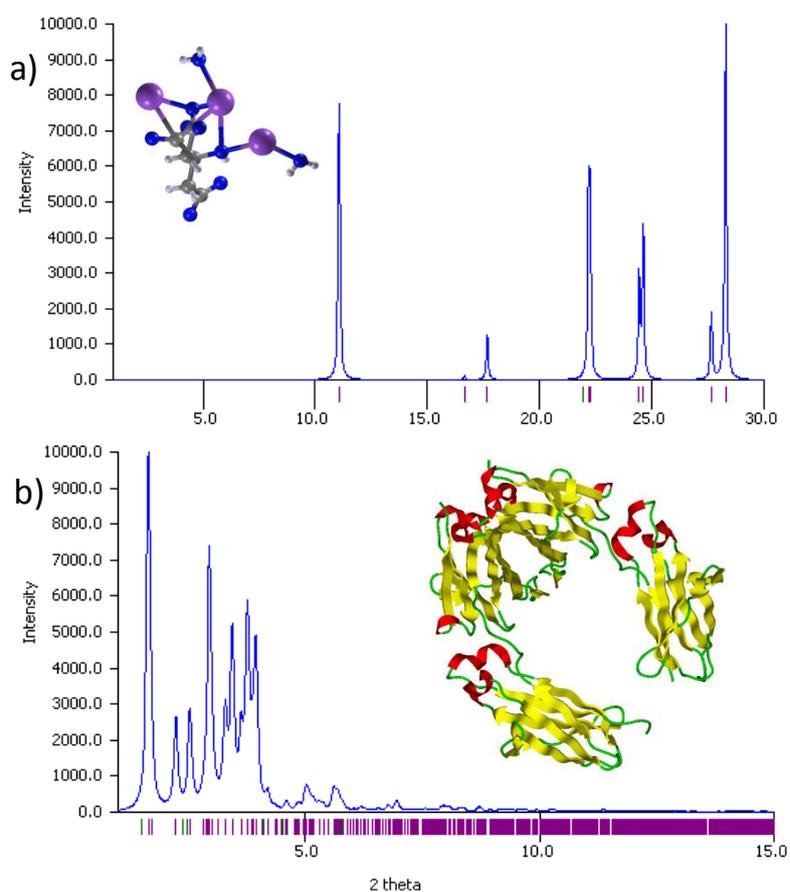
$$2d \sin \theta = n\lambda \quad (\text{S1})$$

high values of the inter-planar distances ( $d$ ), which are typical of large crystal cells, are related to low values of the angle between the diffracted and the primary X-ray beam ( $2\theta$ ). This results in a significant shift of the low resolution limit, i.e. the minimum value of  $2\theta$  is lower in the case of macromolecules. Another peculiarity of powder samples with large unit cell is the huge overlap of diffraction peaks along the  $2\theta$  axis, which increases at higher resolution, as can be deduced by differentiating eq. (S1):

$$\delta\theta \sim \frac{n\lambda}{2d^2} \delta d \quad (\text{S2})$$

Moreover, high resolution limit is limited by the high atomic thermal motion in the case of macromolecules. As an example, in Figure S2 the powder diffraction profile of a small molecule (CCDC code 1478189, Trisodium citrate, unit cell parameters  $a = 7.35 \text{ \AA}$ ,  $b = 5.43 \text{ \AA}$ ,  $c = 11.03 \text{ \AA}$ ) is compared with that of a macromolecule (Fc fragment from human

immunoglobulin G1, PDB code 3AVE, unit cell parameters  $a = 49.42 \text{ \AA}$ ,  $b = 78.47 \text{ \AA}$ ,  $c = 143.76 \text{ \AA}$  [2], both calculated at the same wavelength ( $\lambda = 2.06642 \text{ \AA}$ ). In the case of the small-unit-cell sample (Figure S2 top), diffraction peaks do not show significant reduction up to  $2\theta = 30^\circ$  and they are absent at low resolution ( $2\theta < 10^\circ$ ). Conversely, diffraction peaks populate the low-resolution region and significantly decrease at higher resolution in the case of the large-unit-cell sample (Figure S2 bottom), as it occurs in our sample. This behavior is compatible only with crystals made by macromolecules, where a larger unit cell is needed to host larger molecular size.



**Figure S2** Powder diffraction profiles calculated at the same wavelength ( $2.06642 \text{ \AA}$ ) from the crystal structure of (a) sodium citrate (CCDC code 1478189) and (b) Fc fragment of human immunoglobulin G1 (PDB code 3AVE). Molecules are shown in stick-and-ball (a) and cartoon (b) representations. Diffraction reflections are indicated by lines below the  $2\theta$  axis.

## Reference

[1] a) Von Dreele RB, Stephens PW, Smith GD, Blessing RH, The first protein crystal structure determined from high-resolution X-ray powder diffraction data: a variant of T3R3 human insulin-zinc complex produced by grinding, *Acta Crystallogr D Biol Crystallogr.* 2000, 56(Pt 12), 1549-53.; b) Margiolakia I and Wrighta JP, powder crystallography on macromolecules, *Acta Cryst.* 2008, A64, 169-180.

[2] Matsumiya S, Yamaguchi Y, Saito JI, Nagano M, Sasakawa H, Otaki S, Satoh M, Shitara K, Kato K. Erratum: Structural comparison of fucosylated and nonfucosylated Fc fragments of human immunoglobulin G1. *J. Mol. Biol.* 2011; 386-408.

## Crystal-cell parameters calculation

The algebraic expression which gives the spacing of the lattice planes, identified by the Miller indices  $(hkl)$ , in the hexagonal crystal system is:

$$\frac{1}{d_{hkl}^2} = \frac{4}{3a^2} (h^2 + k^2 + hk) + \frac{l^2}{c^2} \quad (\text{S3})$$

where  $a$  and  $c$  are the crystal cell parameters [1]. By knowing the 1HZH crystal structure [2], it is possible to assign the first two peaks in the generated powder diffraction profile to reflections (300) and (003), respectively. For these two reflections eq. (S3) can be written as

$$d_{300} = \frac{a}{2\sqrt{3}} \quad (\text{S4})$$

$$d_{003} = \frac{c}{3} \quad (\text{S5})$$

Under the hypothesis that the anti-CD20 crystal has the same symmetry and packing of the 1HZH crystal, eq. (S4) and (S5) can be inverted to calculate the cell parameters. By using the resolution values associated to the first two peaks in the experimental powder diffraction profile ( $d_{300} = 66 \text{ \AA}$  and  $d_{003} = 57 \text{ \AA}$ ) we found  $a = 229 \text{ \AA}$  and  $c = 171 \text{ \AA}$ . It is worth noting that the possibility to derive the cell parameters from the position of only two peaks is offered by the high symmetry of the 1HZH crystal cell (H 3 2, belonging to the hexagonal crystal system).

As a validation, we can then use the third 1HZH peak, due to reflection  $(5 \bar{2} 1)$ , to check the assigned values of the cell parameters. For this reflection eq. (S3) becomes  $d_{5\bar{2}1} = \frac{ac\sqrt{3}}{\sqrt{3a^2+76c^2}}$ , which gives  $d_{5\bar{2}1} = 44 \text{ \AA}$  by using the previously determined values of  $a$  and  $c$ . This  $d_{5\bar{2}1}$  value is very close to the experimental value of  $45 \text{ \AA}$  found for the resolution of the third peak.

## References

[1] Giacovazzo C. Fundamentals of crystallography. Oxford university press, New York: 2002.

[2] Saphire EO, Parren PWHI, Pantophlet R, Zwick MB, Morris GM, Rudd PM, Dwek RA, Stanfield RL, Burton DR, Wilson IA. Crystal structure of a neutralizing human IgG against HIV-1: A template for vaccine design. *Science* 2001; 293:1155–1159.