ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Biophysical characterization of the interaction between the full-length XIAP and Smac/DIABLO

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:
Polykretis P., Luchinat E. (2021). Biophysical characterization of the interaction between the full-length XIAP and Smac/DIABLO. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 568(3 September 2021), 180-185 [10.1016/j.bbrc.2021.06.077].

Availability:
This version is available at: https://hdl.handle.net/11585/863482 since: 2022-02-22

Published:
DOI: http://doi.org/10.1016/j.bbrc.2021.06.077

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

12 October 2024

This is the final peer-reviewed accepted manuscript of:
Panagis Polykretis, Enrico Luchinat,
Biophysical characterization of the interaction between the full-length XIAP and Smac/DIABLO,
Biochemical and Biophysical Research Communications, Volume 568, 2021, Pages 180-185, ISSN 0006-291X
The final published version is available online at:
https://doi.org/10.1016/j.bbrc.2021.06.077

Terms of use:
Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)
When citing, please refer to the published version.

Biophysical characterization of the interaction between the full-length XIAP and Smac/DIABLO

Panagis Polykretis [a*], Enrico Luchinat [a,b*]

[a] CERM - Magnetic Resonance Center, University of Florence, via Luigi Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy.
[b] Neurofarba Department, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Italy.

Correspondence email: polykretis@cerm.unifi.it; eluchinat@cerm.unifi.it

Abstract
XIAP is multi-functional protein which regulates apoptosis acting as a direct caspase inhibitor. It is overexpressed in cancer cells, where it antagonizes the pro-apoptotic action of chemotherapeutics, and therefore it has become an important target for the treatment of cancer. In cells undergoing programmed cell death, the pro-apoptotic protein Smac is released by the mitochondria and binds to XIAP, thereby blocking caspase inhibition. Thus, Smac is considered a master regulator of apoptosis in mammals. In this regard, several Smac mimetic compounds have been developed to inhibit XIAP activity in cancer tissues. These compounds have shown low efficacy, partly due to the lack of structural knowledge of the XIAP-Smac interaction. In this work, through SEC-MALS and Circular Dichroism, we provide the first biophysical characterization of the interaction between the full-length form of XIAP and Smac, determining the stoichiometry of the complex and providing important information to develop more effective XIAP inhibitors.

Keywords
XIAP; SmacDIABLO; Protein-protein interaction; SEC-MALS; Circular dichroism

1. Introduction

The X-chromosome linked Inhibitor of Apoptosis Protein (XIAP) is a multifunctional multidomain cytoplasmic protein, which was first recognised as a potent inhibitor of apoptosis [1,2]. XIAP is composed by three N-terminal zinc-binding BIR domains, a ubiquitin associated UBA domain and a C-terminal zinc-binding RING domain [3]. XIAP exerts its anti-apoptotic activity binding and inhibiting the effector caspases 3 and 7 through its BIR2 domain [4–6], and the initiating caspase 9 through its BIR3 domain [7]. When the cell has to undergo programmed cell death in response to apoptotic stimuli, such as DNA damage and the activation of cell-surface death receptors, the caspase cascade is activated by the release of cytochrome *c* from the intermembrane space of mitochondria [8]. Cytochrome *c* associates with Apaf-1 inducing its oligomerization, which in turn leads to the maturation of caspase 9 and consequently of the effector caspase 3 [8]. Another important protein involved in the regulation of apoptosis released by mitochondria, is Smac (second mitochondria-derived activator of caspases) also called DIABLO [9,10]. Smac

eliminates the inhibitory effect of many proteins belonging to the IAP family, and thus it is considered a master regulator of apoptosis in mammals [9,10]. More precisely, previous data show that the wild type mature form of Smac (residues 56-2391-184) interacts with the BIR domains of the proteins belonging to the IAP family through the N-terminal tetrapeptide (A-V-P-I), exerting their inhibitory effects on caspases [11]. XIAP is overexpressed in several types of cancer, in which it potentiates cell survival and resistance to the pro-apoptotic chemotherapeutic drugs [12,13]. Therefore, XIAP has become a promising target for the development of new anticancer treatments [12,14]. Specifically, the class of Smac mimetics, i.e., molecules that mimic the interaction of the AVPI tetrapeptide with the BIR2 and BIR3 domains of XIAP, have been extensively studied [15–19]. However, several Smac mimetics taken into account display a reduced binding affinity for XIAP [13]. The first crystallographic structures showed that Smac forms a dimer which interacts with the BIR2 and BIR3 domains of XIAP [11,20]. However, later on through gel filtration analysis it has been estimated that in solution Smac behaves like an oligomer of approximately 100 kDa [9]. In accordance, a recent structural analysis obtained combining small-angle X-ray scattering (SAXS) and light scattering with molecular modelling concluded that Smac behaves like a flexible tetramer in solution [21]. Furthermore, it has been also shown that Smac has an enhanced affinity for the BIR2-BIR3 construct with respect to the isolated domains [22]. However, the interaction of Smac with the wild type full-length XIAP has never been described, as the full-length form has only recently been purified [3]. In our previous work, we demonstrated that full-length XIAP in solution forms a compact homodimer of 114 kDa, where each subunit – composed of five folded domains separated by unstructured linkers – retains a certain degree of internal flexibility [3]. Consequently, in order to design even more potent IAP inhibitors, it is fundamental to determine the exact stoichiometry of the interaction between XIAP and Smac as it takes place in the cell, considering the physiologically active forms of both partners. In this study, we provide the first biophysical characterization of the interaction between Smac and full-length XIAP, through size exclusion chromatography combined with multi angle light scattering (SEC-MALS) and circular dichroism (CD) analysis.

2. Materials and Methods

2.1 Protein expression and purification

2.1.1 XIAP

Recombinant full-length XIAP was expressed and purified following an existing protocol [3]. Briefly, the gene encoding full-length wild type XIAP (a.a. 1-497) was cloned in the pDEST-His-MBP vector, which adds a GSF sequence at the N-terminus of the protein after cleavage of the tag(computed MW: 56845 Da). The plasmid was used to transform *E. coli* BL21 (DE3) Codon Plus RIPL cells, which were grown in LB medium supplemented with 100 µM $ZnSO_4$ at 37 °C and 170 rpm until mid-log phase. The expression was then induced with 750 µM IPTG, and the cells were grown overnight at 20° C and 170 rpm. The cells were then harvested and re-suspended in 100 mL binding buffer (20 mM Tris, 1 mM of TCEP and

5 mM of Imidazole, pH 8) supplemented with protease inhibitor tablets (Sigma-Aldrich) and lysed by sonication (3 seconds ON, 9 seconds OFF, at 60% of amplitude for 40 minutes). The clarified lysate was passed through a 5 mL HisTrap FF column (Cytiva) and the protein was eluted with the elution buffer (20 mM Tris, 1 mM of TCEP and 500 mM of Imidazole, pH 8). The N-terminal His-MBP tag was cleaved incubating overnight with TEV in dialysis (5 L). As final purification step, XIAP was passed through a size exclusion HiLoad 16/600 Superdex 200 pg chromatography column (GE healthcare Life Sciences) and was transferred in the final buffer (20 mM Tris, 0.5 mM TCEP, pH 7.4).

2.1.2 Smac/DIABLO

The gene encoding the mature form of wild type Smac (a.a. 56-239) was cloned in a pET-28b(+) vector which adds a methionine residue at the N-terminal and a LEHHHHHH tail at the C-terminal of the protein (computed MW: 21961 Da). The plasmid was used to transform *E. coli* BL21 (DE3) cells which were grown in LB medium at 37 °C and 170 rpm. At mid-log phase the expression was induced with 750 µM IPTG and the cell culture was grown for 5 hours at 37° C and 170 rpm. The cells were then harvested and re-suspended in 100 mL binding buffer (20 mM Tris, 1 mM of TCEP and 5 mM of Imidazole, pH 8) supplemented with protease inhibitor tablets (Sigma-Aldrich) and lysed by sonication (3 seconds ON, 9 seconds OFF, at 60% of amplitude for 40 minutes). The clarified lysate was passed through a 5 mL HisTrap FF column (Cytiva) and the protein was eluted with the elution buffer (20 mM Tris, 1 mM of TCEP and 500 mM of Imidazole, pH 8). Finally, Smac was passed through a size exclusion HiLoad 16/600 Superdex 200 pg column (GE healthcare Life Sciences) and was transferred in the same final buffer of XIAP (20 mM Tris, 0.5 mM TCEP, pH 7.4).

2.2 SEC-MALS

SEC-MALS experiments were performed using a Superdex 200 10/300 GL column (GE healthcare Life Sciences) at a flowrate of 0.75 mL/min. The instrument setup includes a multi-angle light scattering (DAWN EOS, Wyatt Technology Corp.) with a quasi-elastic light scattering detector (QUELS, Wyatt Technology) and a refractometer with extended range (Optilab rEX, Wyatt Technology Corp.), connected to a high-performance liquid chromatographer. The data were analysed with the ASTRA V (Wyatt Technology Corp.) software.

2.3 Analytical gel filtration

The analytical gel filtration experiments were performed using a Superdex 200 Increase 10/300 GL column (GE healthcare Life Sciences) injecting 500 µL of protein sample and eluting at 0.75 mL/min flowrate with 20 mM Tris, 0.5 mM TCEP, pH 7.4 buffer. The elution fractions were collected and analysed by SDS-PAGE using Any kD™ Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad).

2.4 Circular Dichroism

CD experiments were performed on a JASCO J-810 spectrometer using a 0.1 cm quartz cuvette containing 400 µL of protein solution. The spectra were recorded over wavelength range 200-250 nm at 25° C. The unsmoothed spectra are the average of 5 scans and were processed with the JASCO Spectra Manager software. The calculation of secondary structures was performed using the BeStSel (Beta Structure Selection) web tool [23,24].

3. Results

3.2 SEC-MALS

SEC-MALS is an efficient technique for the characterization of protein mass, interactions, oligomerization and aggregation state [25]. SEC-MALS analysis performed on Smac allowed us to determine that in solution the protein forms a monodisperse species with an average experimental molecular weight (MW_{avg}) of 88 kDa (uncertainty 0.1%), which is compatible with a tetrameric oligomerization state (**Fig. 1a**). Notably, the reported MW_{avg} is slightly lower than that previously reported by gel filtration (~100 kDa [9]) and by SAXS and static light scattering experiments (90 kDa [21]), and is in excellent agreement with the theoretical MW (87.8 kDa). SEC-MALS analysis of XIAP is consistent with our previous finding [3], and confirms that XIAP in solution forms a homodimer of MW_{avg} of 115 kDa (uncertainty <0.1%, theoretical MW: 113.7 kDa) (**Fig. 1b**). Interestingly, the tetramer of Smac elutes faster from the size exclusion column than the dimer of XIAP, even if it has a lower molecular weight, thus explaining the higher MW previously reported by gel filtration analysis. As a matter of fact, the conformational properties of each macromolecule can affect the elution speed within the pores of the resin, and thus can happen that a protein with a stretched conformation elutes earlier than a bigger, but more compact one.

Since Smac forms a tetramer in solution, we mixed 10 µM of XIAP (dimer concentration) with 10 µM of Smac (tetramer concentration) and we performed SEC-MALS analysis on the mixture. Such experiment demonstrated that the two proteins interact with a 2:4 monomer stoichiometry (i.e., 1 XIAP dimer : 1 Smac tetramer) forming a single monodisperse species with a MW_{avg} of 209 kDa (uncertainty 0.1%), which is very close to the theoretical molecular weight of the complex (201.5 kDa) (**Fig. 1c**). Mixing XIAP and Smac with a 2:1 monomer ratio resulted in two distinct species in the chromatogram: one peak can be attributed to the 2:4 complex (MW_{avg} 224 kDa, uncertainty 0.1%) and one belongs to the homodimer of XIAP (MW_{avg} 110 kDa, uncertainty 0.2%) (**Fig. 1d**). Finally, mixing XIAP and Smac with a 1:10 monomer ratio resulted in a single broad peak in the chromatogram (**Fig. 1e**). Analysis of the light scattering data revealed that the peak has two components with different scattering profiles, due to the overlap between the elution peak of the Smac with that of the complex, with MW_{avg} of 204 and 100 kDa (uncertainty 0.1%), consistent with the 2:4 complex and with the Smac tetramer, respectively.

3.2 *Analytical gel filtration*

Analytical gel filtration experiments confirmed the results obtained through SEC-MALS. Both Smac and XIAP are monodisperse in solution and their elution peaks resulted in a single band at ~22 and ~57 kDa, respectively, when run on a denaturing and reducing SDS-PAGE **(Fig. 2a**, **b** and **e**). Even in the case of the analytical gel filtration (which was performed on a different column than SEC-MALS), the Smac tetramer eluted slightly earlier with respect to the XIAP dimer, despite the higher molecular weight of the latter. The SDS-PAGE analysis of fraction corresponding to the single elution peak of the complex (**Fig. 2c**) displayed two separated bands (**Fig. 2e**), confirming that the two proteins form a 2:4 complex, which is disrupted under denaturing and reducing conditions. This is in accordance with the fact that Smac interacts with the BIR domains of XIAP through non-covalent interactions [11]. Similarly, the SDS-PAGE of the fractions corresponding to the two peaks obtained by mixing XIAP and Smac in a 2:1 monomer ratio (**Fig. 2d**) confirmed that they belong to the 2:4 complex and to XIAP, respectively (**Fig. 2e**).

3.3 *Circular Dichroism*

Far ultraviolet (UV) CD spectroscopy is a frequently used technique for the assessment of protein secondary structure in solution. Each protein has an intrinsic CD spectrum arising from the amide backbone, which absorbs in the Far-UV. CD analysis performed over a wavelength range of 200-250 nm showed that both Smac and XIAP are folded at 25° C, temperature at which all the experiments in this study were performed. The secondary structure contents of the proteins were estimated by fitting the CD experimental data with BeStSel [23,24]. The CD spectrum of the Smac tetramer was best fitted with 57% α-helix and 25.8% random coil, with minor amounts of other secondary structures (**Fig. 3a**). The CD spectrum of the XIAP dimer was best fitted with 23.8% α-helix, 17.4% β-sheet, 16.3% turn and 42.5% random coil (**Fig. 3b**). CD spectra were then recorded on the complex formed by XIAP and Smac, to assess whether the complex formation causes changes in secondary structure composition. As expected, the contribution of Smac in the increase of the α-helical content of the complex is clearly visible from the shape of the resulting CD spectrum, where it is evident a decrease of ellipticity at 208 and 222 nm, typical of the increment of α-helix [26,27] (**Fig. 3c**). Such increment is confirmed even by the fitting which indicates that the complex is composed by 41.5% α-helix, 9.1% β-sheet, 12.5% turn and 36.9% random coil. In order to monitor the decrease of ellipticity at 208 and 222 nm, XIAP has been titrated with increasing equivalents of Smac, and CD spectra were recorded at each step (**Fig. 3d**). The titration shows that there is a gradual decrement of ellipticity at 208 and 222 nm, proportional to the amount of Smac added to the solution, demonstrating that the formation of the complex does not induce a change in the overall secondary structure.

4. Discussion

XIAP is a fundamental inhibitor of apoptosis in human cells and has been extensively studied in the last two decades [2,12,13]. Many trials were done and are still in progress in order to design efficient inhibitors of XIAP as a treatment of malignancy [12,14–19]. To date,

the most successful approach *in vitro* was based on the inhibition of the interaction between XIAP and Smac by Smac mimetics [15,18]. Nevertheless, up to now all the trials were done on single domains of XIAP separately, such as BIR2 or BIR3, because the full-length form of the protein has only recently been obtained [3]. Furthermore, it has been previously hypothesized that the design of more potent inhibitors should take in account the interaction of the full-length XIAP with a tetramer of Smac [3,21]. This work provides the first biophysical characterization of the interaction between the full-length form of XIAP and Smac. Through biophysical experimental techniques, we first confirmed the previously reported quaternary structures of isolated XIAP and Smac in solution [3,21]. Then, we determined the exact stoichiometry of the interaction, showing that one homodimer of XIAP binds one tetramer of Smac forming a 201.5 kDa complex. We also showed that the interaction does not change the overall secondary structure content of the complex, suggesting that the linkers between the domains of XIAP retain their flexibility in the complex. Notably, complexes with different stoichiometries were not detected in any of the experiments, independent of the molar ratios at which the two proteins were made to interact. This implies that the intrinsic flexibility of the proteins involved does not modulate the oligomeric state of the complex, unlike what previously suggested [21]. These data provide clear and important information about the interaction of these two proteins, and will open the way to further structural characterization of the complex through high-resolution techniques, which is fundamental in order to design more efficient inhibitors of XIAP.

Conflicts of interest/role of the funding source

The authors declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Instruct-ERIC, a Landmark ESFRI project, and specifically by the CERM/CIRMMP Italy Centre.

Figures

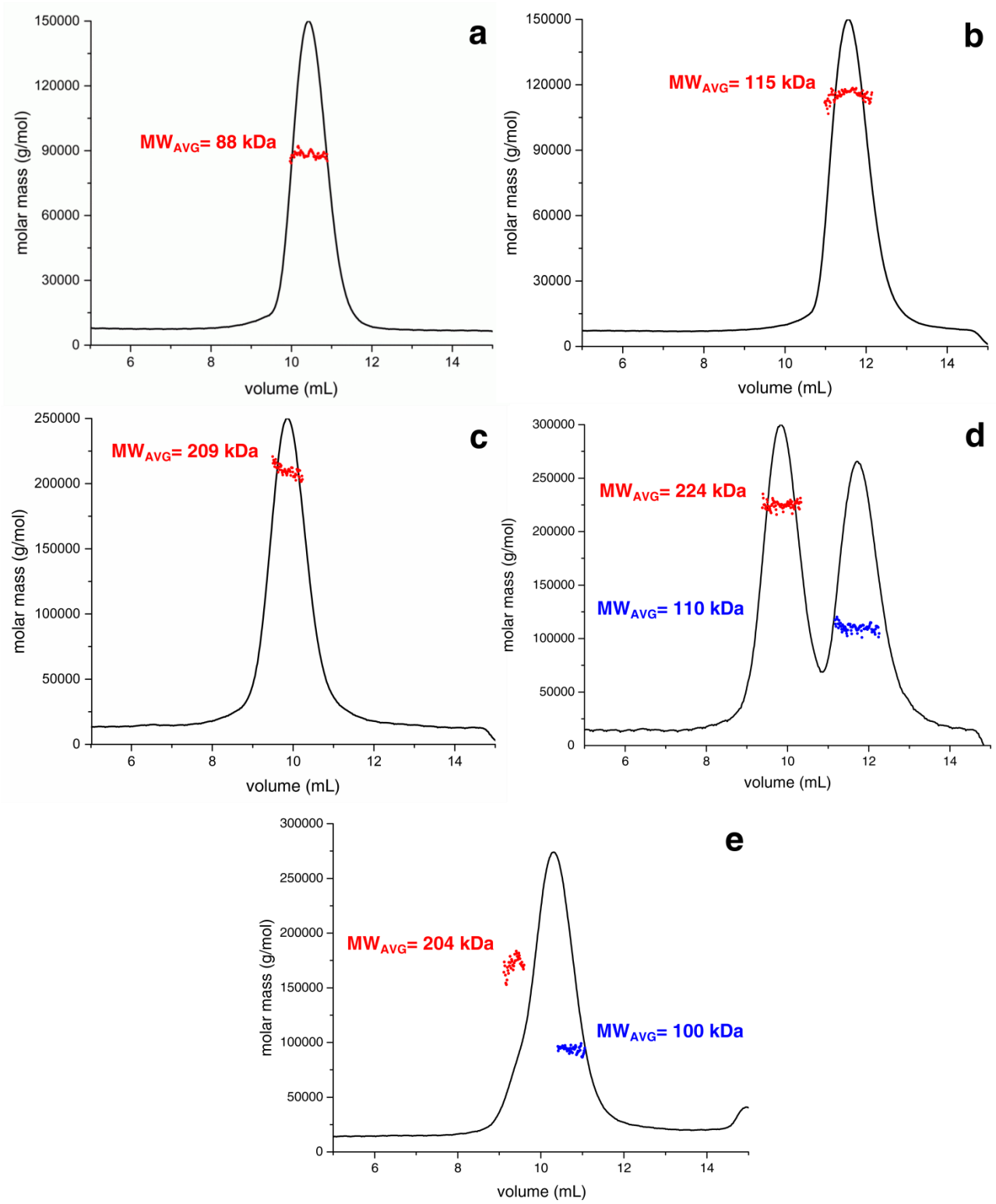

Figure 1: SEC-MALS experiments performed on **a**) Smac 10 µM (tetramer concentration); **b**) XIAP 15 µM (dimer concentration); **c**) XIAP 10 µM (dimer concentration) + Smac 10 µM (tetramer concentration); **d**) XIAP 10 µM (dimer concentration) + Smac 2.5 µM (tetramer concentration); **e**) XIAP 5 µM (dimer concentration) + Smac 25 µM (tetramer concentration).

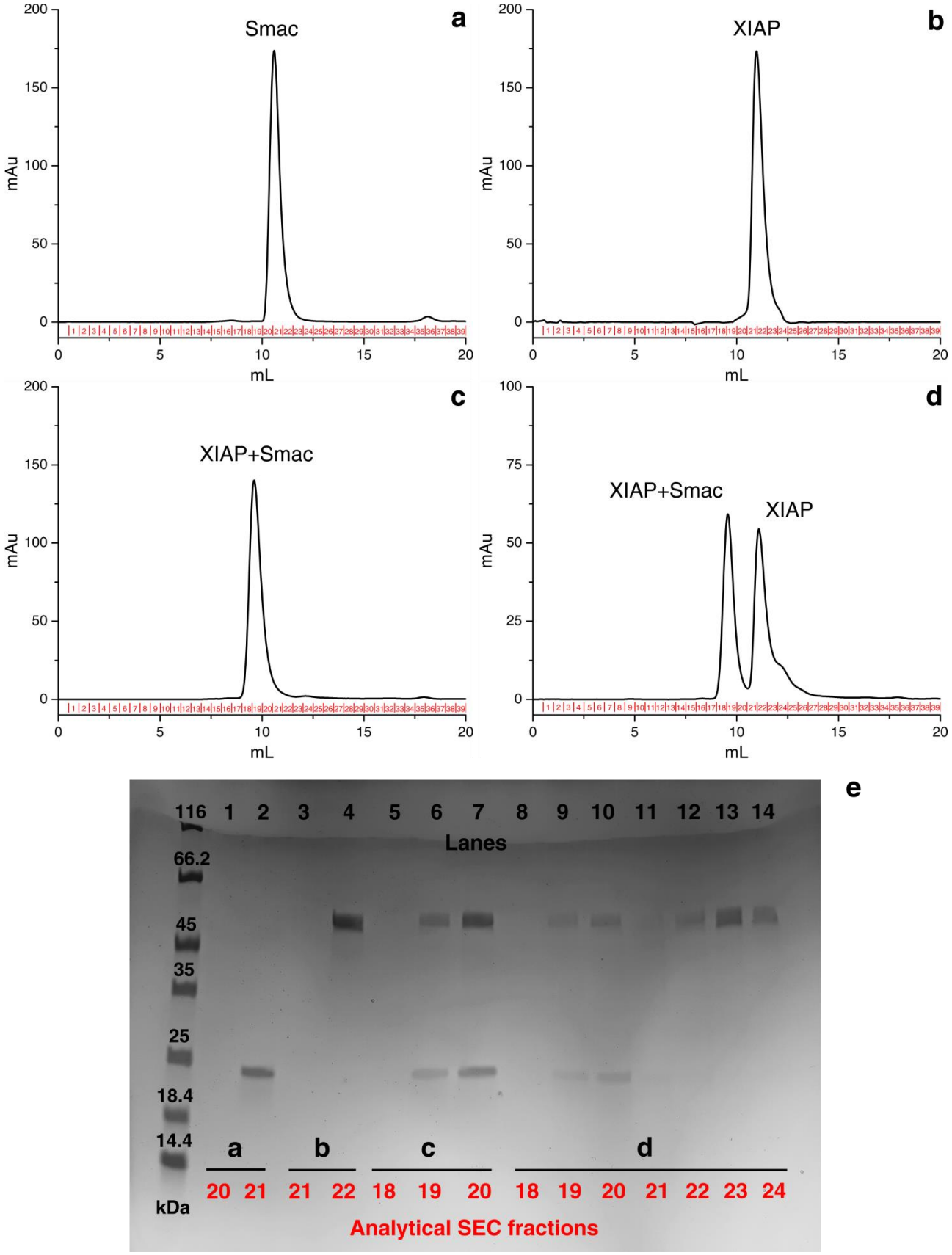

Figure 2: Analytical gel filtration experiments performed on **a**) Smac 10 µM (tetramer concentration); **b**) XIAP 15 µM (dimer concentration); **c**) XIAP 10 µM (dimer concentration) + Smac 10 µM (tetramer concentration); **d**) XIAP 10 µM (dimer concentration) + Smac 2.5 µM (tetramer concentration); **e**) SDS-PAGE analysis performed on the 500 µL elution fractions corresponding to the peaks of the aforementioned chromatographies.

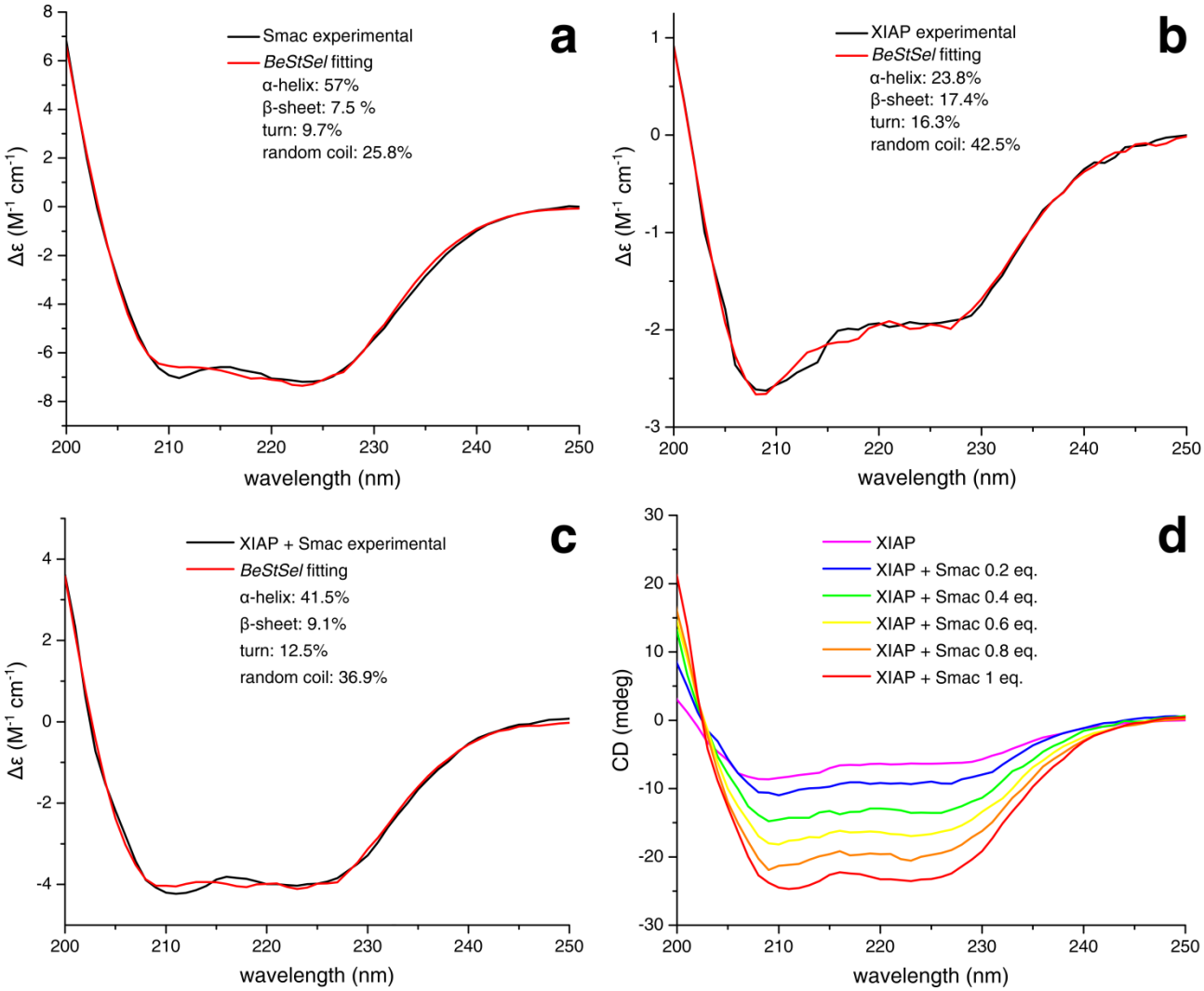

Figure 3: **a**) CD spectrum of Smac 2 µM (tetramer concentration) acquired at 25° C *vs* BeStSel fitting curve (RMSD: 0.168); **b**) CD spectrum of XIAP 2 µM (dimer concentration) acquired at 25° C *vs* BeStSel fitting curve (RMSD: 0.061); **c**) CD spectrum of XIAP 1 µM (dimer concentration) + Smac 1 µM (tetramer concentration) acquired at 25° C *vs* BeStSel fitting curve (RMSD: 0.055); **d**) CD spectrum of XIAP 1 µM (dimer concentration) titrated with increasing concentrations of Smac tetramer, acquired at 25° C.

References

[1] P. Liston, N. Roy, K. Tamai, C. Lefebvre, S. Baird, G. Cherton-Horvat, R. Farahani, M. McLean, J.E. Ikeda, A. MacKenzie, R.G. Korneluk, Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes, Nature, 379 (1996) 349–353.

[2] Q.L. Deveraux, R. Takahashi, G.S. Salvesen, J.C. Reed, X-linked IAP is a direct inhibitor of cell-death proteases, Nature, 388 (1997) 300–304.

[3] P. Polykretis, E. Luchinat, A. Bonucci, A. Giachetti, M.A. Graewert, D.I. Svergun, L. Banci, Conformational characterization of full-length X-chromosome-linked inhibitor of apoptosis protein (XIAP) through an integrated approach, IUCrJ, 6 (2019) 948–957.

[4] C. Sun, M. Cai, A.H. Gunasekera, R.P. Meadows, H. Wang, J. Chen, H. Zhang, W. Wu, N. Xu, S.-C. Ng, S.W. Fesik, NMR structure and mutagenesis of the inhibitor-of-apoptosis protein XIAP, Nature, 401 (1999) 818–822.

[5] S.J. Riedl, M. Renatus, R. Schwarzenbacher, Q. Zhou, C. Sun, S.W. Fesik, R.C. Liddington, G.S. Salvesen, Structural basis for the inhibition of caspase-3 by XIAP, Cell, 104 (2001) 791–800.

[6] J. Chai, E. Shiozaki, S.M. Srinivasula, Q. Wu, P. Datta, E.S. Alnemri, Y. Shi, P. Dataa, Structural basis of caspase-7 inhibition by XIAP, Cell, 104 (2001) 769–780.

[7] S.M. Srinivasula, R. Hegde, A. Saleh, P. Datta, E. Shiozaki, J. Chai, R.A. Lee, P.D. Robbins, T. Fernandes-Alnemri, Y. Shi, E.S. Alnemri, A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis, Nature, 410 (2001) 112–116.

[8] X. Jiang, X. Wang, Cytochrome C-Mediated Apoptosis, Annual Review of Biochemistry, 73 (2004) 87–106.

[9] C. Du, M. Fang, Y. Li, L. Li, X. Wang, Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition, Cell, 102 (2000) 33–42.

[10] A.M. Verhagen, D.L. Vaux, Cell death regulation by the mammalian IAP antagonist Diablo/Smac, Apoptosis, 7 (2002) 163–166.

[11] G. Wu, J. Chai, T.L. Suber, J.-W. Wu, C. Du, X. Wang, Y. Shi, Structural basis of IAP recognition by Smac/DIABLO, Nature, 408 (2000) 1008–1012.

[12] S. Fulda, D. Vucic, Targeting IAP proteins for therapeutic intervention in cancer, Nat Rev Drug Discov, 11 (2012) 109–124.

[13] R. Abbas, S. Larisch, Targeting XIAP for Promoting Cancer Cell Death—The Story of ARTS and SMAC, Cells, 9 (2020) 663.

[14] R. Mannhold, S. Fulda, E. Carosati, IAP antagonists: promising candidates for cancer therapy, Drug Discov. Today, 15 (2010) 210–219.

[15] P. Boddu, B.Z. Carter, S. Verstovsek, N. Pemmaraju, SMAC mimetics as potential cancer therapeutics in myeloid malignancies, British Journal of Haematology, 185 (2019) 219–231.

[16] S. Fulda, Chapter Four - Smac Mimetics to Therapeutically Target IAP Proteins in Cancer, in: L. Galluzzi (Ed.), International Review of Cell and Molecular Biology, Academic Press, 2017: pp. 157–169.

[17] E.J. Hennessy, A. Adam, B.M. Aquila, L.M. Castriotta, D. Cook, M. Hattersley, A.W. Hird, C. Huntington, V.M. Kamhi, N.M. Laing, D. Li, T. MacIntyre, C.A. Omer, V. Oza, T. Patterson, G. Repik, M.T. Rooney, J.C. Saeh, L. Sha, M.M. Vasbinder, et al., Discovery of a Novel Class of Dimeric Smac Mimetics as Potent IAP Antagonists

Resulting in a Clinical Candidate for the Treatment of Cancer (AZD5582), J. Med. Chem., 56 (2013) 9897–9919.
[18] E. Morrish, G. Brumatti, J. Silke, Future Therapeutic Directions for Smac-Mimetics, Cells, 9 (2020) 406.
[19] C. Baggio, L. Gambini, P. Udompholkul, A.F. Salem, A. Aronson, A. Dona, E. Troadec, F. Pichiorri, M. Pellecchia, Design of Potent pan-IAP and Lys-Covalent XIAP Selective Inhibitors Using a Thermodynamics Driven Approach, J. Med. Chem., (2018).
[20] J. Chai, C. Du, J.-W. Wu, S. Kyin, X. Wang, Y. Shi, Structural and biochemical basis of apoptotic activation by Smac/DIABLO, Nature, 406 (2000) 855–862.
[21] E. Mastrangelo, P. Vachette, F. Cossu, F. Malvezzi, M. Bolognesi, M. Milani, The Activator of Apoptosis Smac-DIABLO Acts as a Tetramer in Solution, Biophysical Journal, 108 (2015) 714–723.
[22] Y. Huang, R.L. Rich, D.G. Myszka, H. Wu, Requirement of Both the Second and Third BIR Domains for the Relief of X-linked Inhibitor of Apoptosis Protein (XIAP)-mediated Caspase Inhibition by Smac*, Journal of Biological Chemistry, 278 (2003) 49517–49522.
[23] A. Micsonai, F. Wien, L. Kernya, Y.-H. Lee, Y. Goto, M. Réfrégiers, J. Kardos, Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy, PNAS, 112 (2015) E3095–E3103.
[24] A. Micsonai, F. Wien, É. Bulyáki, J. Kun, É. Moussong, Y.-H. Lee, Y. Goto, M. Réfrégiers, J. Kardos, BeStSel: a web server for accurate protein secondary structure prediction and fold recognition from the circular dichroism spectra, Nucleic Acids Res, 46 (2018) W315–W322.
[25] D. Some, H. Amartely, A. Tsadok, M. Lebendiker, Characterization of Proteins by Size-Exclusion Chromatography Coupled to Multi-Angle Light Scattering (SEC-MALS), J Vis Exp, (2019).
[26] N.J. Greenfield, Circular dichroism analysis for protein-protein interactions, Methods Mol Biol, 261 (2004) 55–78.
[27] N.J. Greenfield, Determination of the folding of proteins as a function of denaturants, osmolytes or ligands using circular dichroism, Nature Protocols, 1 (2006) 2733–2741.