InChI for large molecules Workshop

Supported by:

IUPAC Division VIII InChI subcommittee

NCBI/NLM

InChI Trust

Lister Hill Center Auditorium
National Library of Medicine
Bldg. 38/Lister Hill Center
1st floor Lobby-Auditorium

October 27-28 , 2014

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Use cases

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- Must be quick .. E.g., handle molecules containing up to 15K heavy atoms (1500 residues) in less than one second
- Able to determine the novelty of the chemical entity
- Compare in chemical or sequence based structures
- Can do a search by search engine (e.g., Google)
- Different input formats yields same result (PDB, HELM, SCSR, SMILES, FASTA, MOL/SDF, etc.)
- Can be converted back into output format (PDB, HELM, SCSR, SMILES, FASTA, MOL/SDF, etc.)
- Can handle undefined attachment points of chemical entities (e.g., 1-4 vs. 1-6 in carbohydrates) and variable/undefined stereochemistry (e.g., alpha/beta) and ring open/close variants

Use cases

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- Can handle a range of attachments at a defined set of possible locations (e.g., 3 entities with 5 potential places to go)
- Can handle payloads, mutated and modified residues beyond that handled by FASTA
- Be able to group identifiers by sequence
- Handle stereo center variation (L vs. D) for a large number (up to max supported residues)
- Consider arbitrary limit on molecule size (although may have performance implications)
- Be able to retain original sequence information even if chemically modified to be something else (e.g., covalent bonding modification such as cyclization of peptide side chains, etc.)

Use Cases

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- Be able to represent complex connectivity with metals, e.g., {cysteine} S-Fe clusters
- Be able to handle peptide/saccharide complexes within a larger complex system, e.g., biological interesting molecules dictionary (BIRD – 1000 cases) .. E.g, be able to handle saccharide cases.
- Handle representation of non-standard polymers found in PTMs, peptides, saccharides, chromophores cases
- Consider generic polymer handling (e.g., undefined overall chemical structure but known components or connection points .. no arbitrary restrictions)
- *

Use cases

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- Ensemble molecule with distributions of moieties (e.g., variably described molecule mixture that contains a range of molecular entities that are attached {2-4 of X attached, where X might be a peptide chain})
- Capturing oxidation state of metals complexed with proteins or in nanoparticles
- Must handle well defined large molecules
- Can handle RNA/DNA (nucleic acids) and other biopolymer types that are well defined
- Ability to handle well-defined quat-structure (non-covalently bound, e.g., hemoglobin but not insulin)
- Attempt to preserve stoichiometry of the moieties in question

Use cases

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- Ability to ignore hydration from chemistry/sequence description
- Ignore polymorphs (except if stoichiometry is different, do not ignore)
- Consider PEG-ylation aspects (e.g., of proteins and peptides)
- Ability to cover most biopharmaceuticals that are marketed drugs (as-is possible)
- Must be able to handle drugs like defibrotide, heparin
- Handle lipid nanoparticles (e.g., lipidsomes)
- Can handle isotopes (consider cases of variable isotopic enrichment)

High level use cases

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- Chemically Modified Biologics exhibit many challenges in chemical representation
  - Size
  - Variable substitution sites
  - Variable substitution loading
  - Hydrogen bonding
  - Presence of heavy metals

Biopolymer testing with InChI v1.05

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Background

- Initial releases of InChI were limited to 1024 heavy atoms
- Many biopolymers of interest contain more than 1024 heavy atoms
- v1.05 removes this limitation and enables InChIs and InChI keys to be calculated for large structures
- This presentation summarizes initial work with large structures using a pre-release version of the software
- The Winchi-1.exe was used to calculate the InChI keys
- Filgrastim sequence was used as the basis for most of the experiments

Limitations

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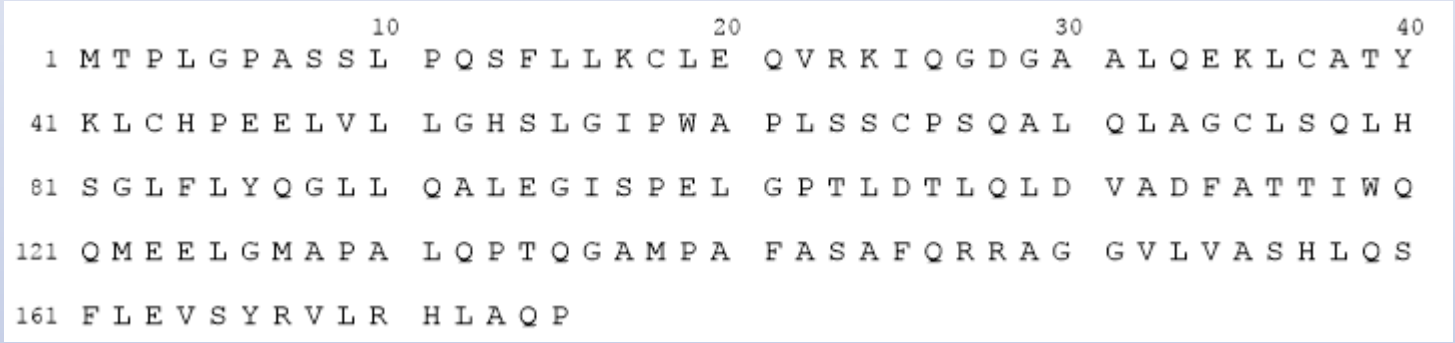
- Structures must be in molfile format
  - V2000 and v3000 formats are accepted
    - v3000 is required for large structures
- The Self Contained Sequence Representation (SCSR) is not supported yet
- Sgroups are not supported and must be removed before presentation to the InChI code
  - Many biopolymer structures contain Sgroup features by default
  - Removal can be achieved programmatically or by editing the molfile in a text editor

Large structure

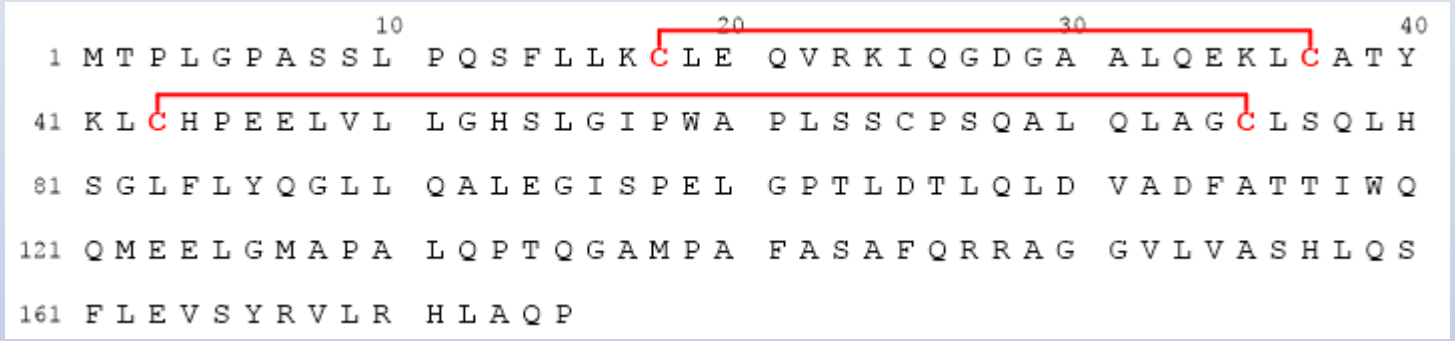
- Filgrastim



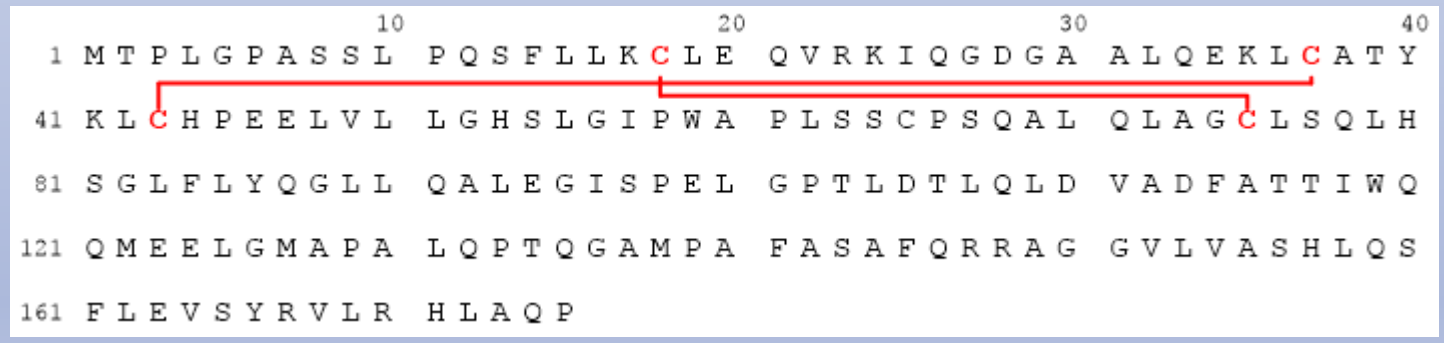
- InChIKey=KOKXRWZWQJXBOP-NJDFSSKJBA-N

With disulfide bridges

- InChIKey=MMCZGSMNPYTOPN-NJDFSSKJBA-N

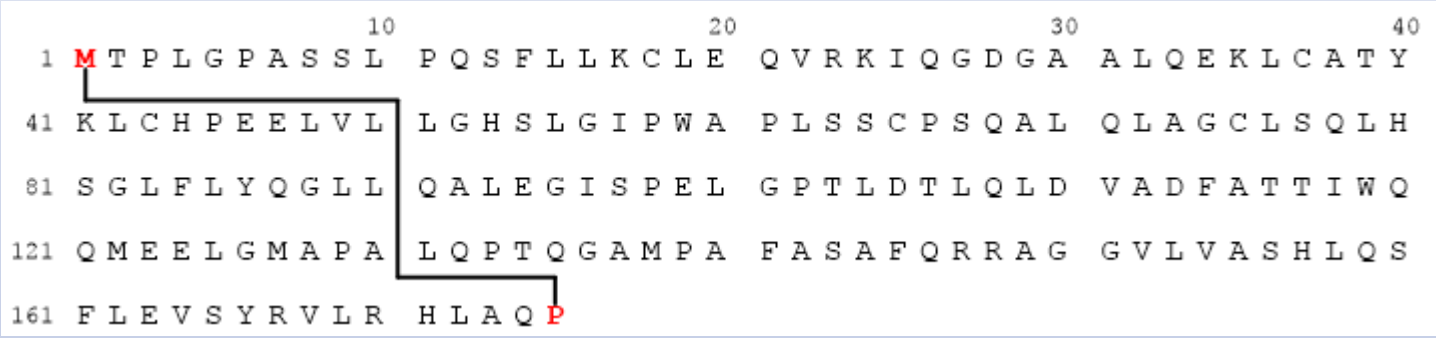


- InChIKey=MEMBSBQMAVSGHQ-NJDFSSKJBA-N

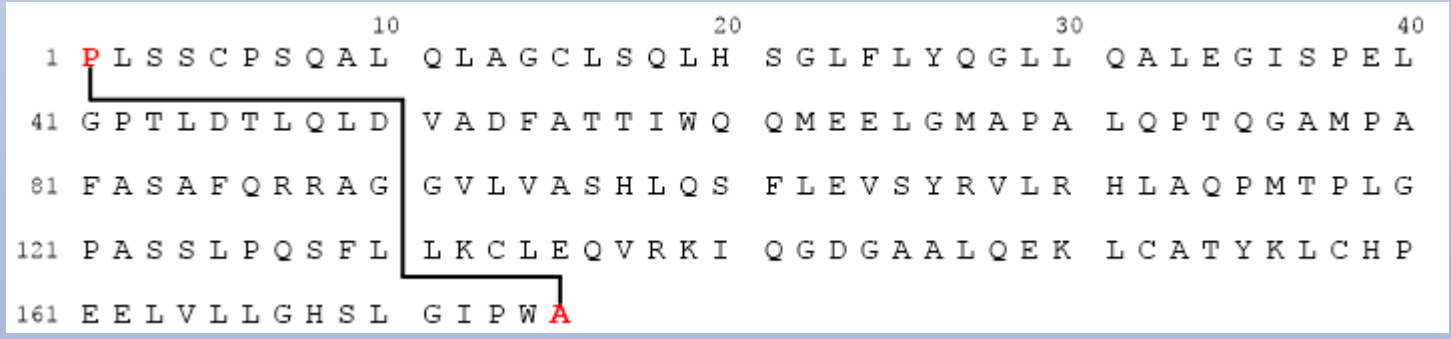


Cyclized

- InChIKey=IZNXXFOUFDSLAX-VBNFVGOYBA-N

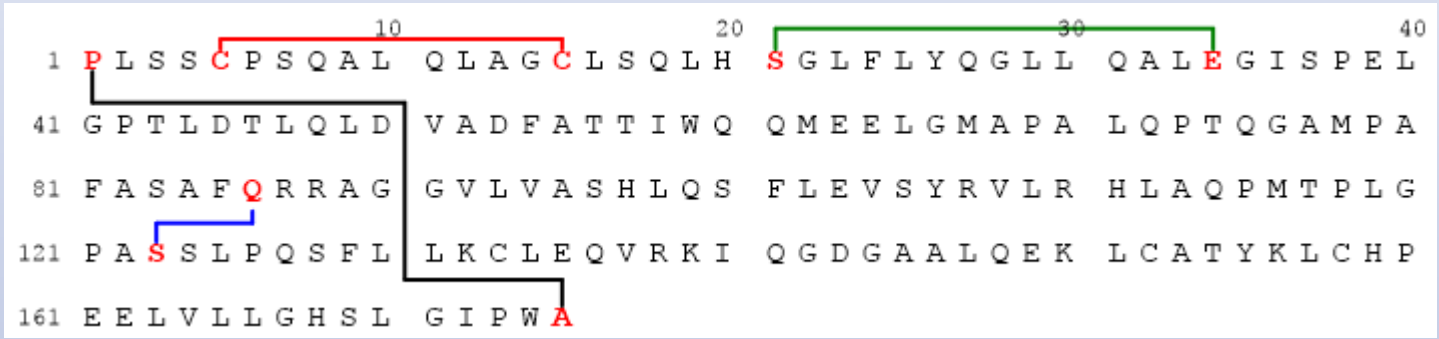


- InChIKey=IZNXXFOUFDSLAX-VBNFVGOYBA-N



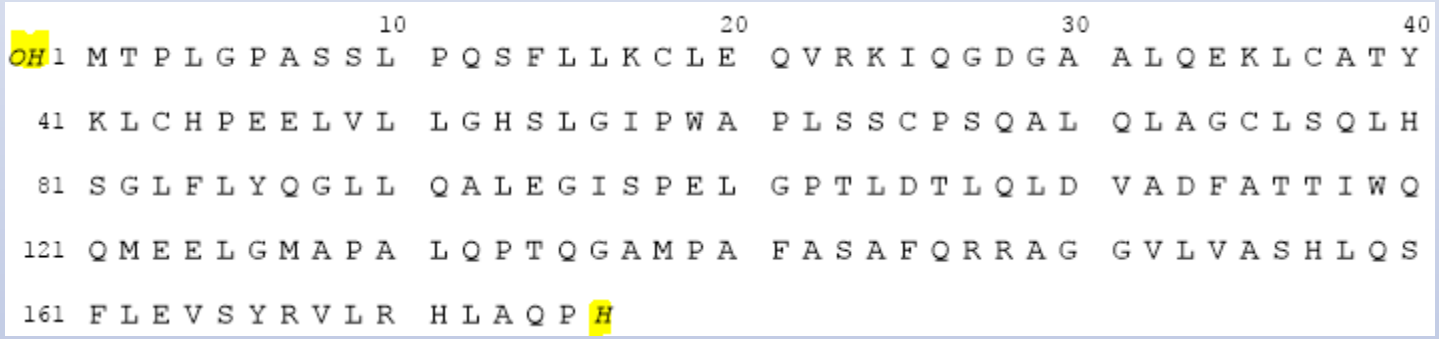
Multiple cyclizations

- InChIKey=AQUGLJGKXYTOSD-VBNFVGOYBA-N



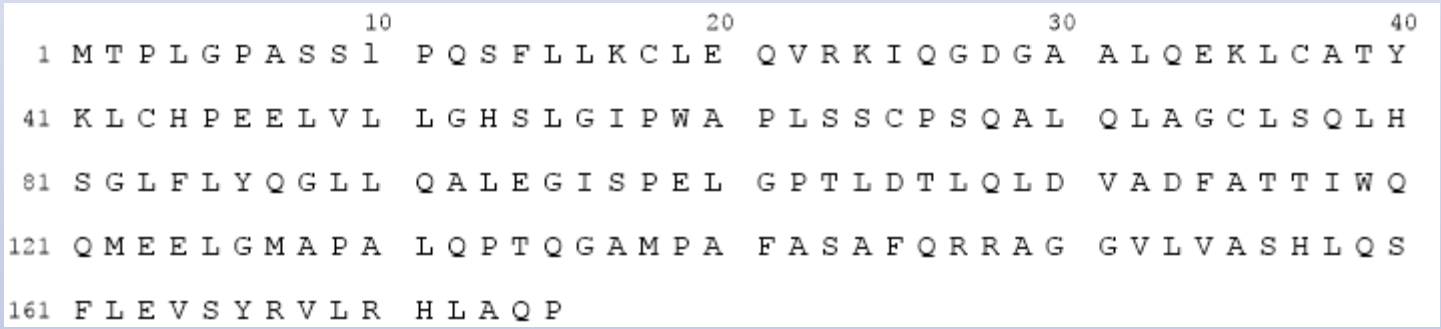
Reversed sequence

- InChIKey=YFXNVYXMKDIHRN-VBNFVGOYBA-N



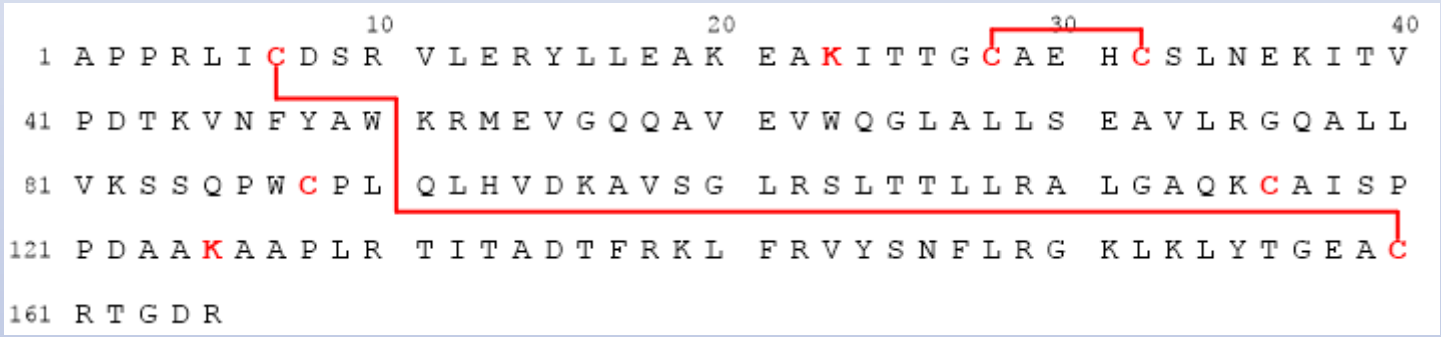
Filgrastim Lys10-D form

- InChIKey=KOKXRWZWQJXBOP-FNWNWACTBA-N



Synthetic Erythropoetin

- InChIKey=XJBDLLBKVUYAKW-WAXLMBMOBA-D



- PEGylated at K23 and K125
- Acylated at C88 and C106

Polynucleotide - 1

- InChIKey=NELTZQNSFHRPGO-AZBJDUHQBA-N

- Calculation time: ~6s
- Molecular Formula: $C_{2894}H_{3649}N_{1147}O_{1791}P_{300}$

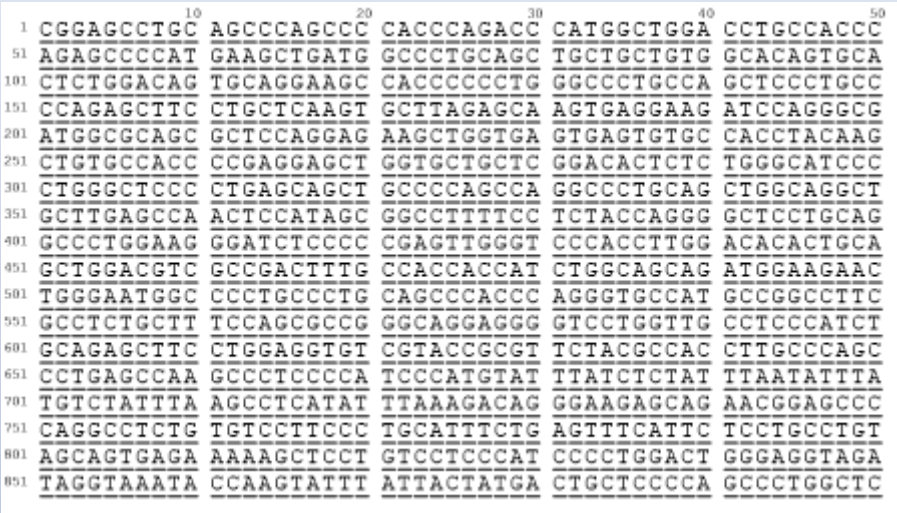
Polynucleotide - 2

- InChIKey=IHDBOWIRPNDUCX-YUQJSOSJBA-N

- Calculation time: ~38s
- Molecular Formula: $C_{5782}H_{7305}N_{2255}O_{3602}P_{600}$

Polynucleotide - 3

- InChIKey=



- Calculation timeout at ~125s
- Molecular Formula: $C_{8693}H_{10989}N_{3325}O_{5420}P_{900}$

Myosin-1

- InChIKey=BBJMARUZQDWUQG-PZLOAVSTBA-N

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   1 MSSDSEMAIF GEAAPFLRKS ERERIEAQNK PFDAKTSVFV VDPKESFVKA
  51 TVQSREGGKV TAKTEAGATV TVKDDQVFPM NPPKYDKIED MAMMTHLHEP
 101 AVLYNLKERY AAWMIYTYSG LFCVTVNPYK WLPVYNAEVV TAYRGKKRQE
 151 APPHIFSISD NAYQFMLTDR ENQSILITGE SGAGKTVNTK RVIQYFATIA
 201 VTGEKKKEEV TSGKMQGTLE DQIISANPLL EAFGNAKTVR NDNSSRFGKF
 251 IRIHFGTTGK LASADIETYL LEKSRVTFQL KAERSYHIFY QIMSNKKPDL
 301 IEMLLITTNP YDYAFVSQGE ITVPSIDDQE ELMATDSAIE ILGFTSDERV
 351 SIYKLTGAVM HYGNMKFKQK QREEQAEPDG TEVADKAAYL QNLNSADLLK
 401 ALCYPRVKVG NEYVTKGQTV QQVYNAVGAL AKAVYDKMFL WMVTRINQQL
 451 DTKQPRQYFI GVLDIAGFEI FDFNSLEQLC INFTNEKLQQ FFNHHMFVLE
 501 QEEYKKEGIE WTFIDFGMDL AACIELIEKP MGIFSILEEE CMFPKATDTS
 551 FKNKLYEQHL GKSNNFQKPK PAKGKPEAHF SLIHYAGTVD YNIAGWLDKN
 601 KDPLNETVVG LYQKSAMKTL ALLFVGATGA EAEAGGGKKG GKKKGSSFQT
 651 VSALFRENLN KLMTNLRSTH PHFVRCIIPN ETKTPGAMEH ELVLHQLRCN
 701 GVLEGIRICR KGFPSRILYA DFKQRYKVLN ASAIPEGQFI DSKKASEKLL
 751 GSIDIDHTQY KFGHTKVFFK AGLLGLLEEM RDEKLAQLIT RTQAMCRGFL
 801 ARVEYQKMVE RRESIFCIQY NVRAFMNVKH WPWMKLYFKI KPLLKSAETE
 851 KEMANMKEEF EKTKEELAKT EAKRKELEEK MVTLMQEKND LQLQVQAEAD
 901 SLADAEERCD QLIKTKIQLE AKIKEVTERA EDEEEINAEL TAKKRKLEDE
 951 CSELKKDIDD LELTLAKVEK EKHATENKVK NLTEEMAGLD ETIAKLTKEK
1001 KALQEAHQQT LDDLQAEEDK VNTLTKAKIK LEQQVDDLEG SLEQEKKIRM
1051 DLERAKRKLE GDLKLAQEST MDIENDKQQL DEKLKKKEFE MSGLQSKIED
1101 EQALGMQLQK KIKELQARIE ELEEEIEAER ASRAKAEKQR SDLSRELEEI
1151 SERLEEAGGA TSAQIEMNKK REAEFQKMRR DLEEATLQHE ATAATLRKKH
1201 ADSVAELGEQ IDNLQRVKQK LEKEKSEMKM EIDDLASNME TVSKAKGNLE
1251 KMCRALEDQL SEIKTKEEEQ QRLINDLTAQ RARLQTESGE YSRQLDEKDT
1301 LVSQLSRGKQ AFTQQIEELK RQLEEEIKAK SALAHALQSS RHDCDLLREQ
1351 YEEEQEAKAE LQRAMSKANS EVAQWRTKYE TDAIQRTEEL EEAKKKLAQR
1401 LQDAEEHVEA VNAKCASLEK TKQRLQNEVE DLMIDVERTN AACAALDKKQ
1451 RNFDKILAEW KQKCEETHAE LEASQKESRS LSTELFKIKN AYEESLDQLE
1501 TLKRENKNLQ QEISDLTEQI AEGGKRIHEL EKIKKQVEQE KSELQAALEE
1551 AEASLEHEEG KILRIQLELN QVKSEVDRKI AEKDEEIDQM KRNHIRIVES
1601 MQSTLDAEIR SRNDAIRLKK KMEGDLNEME IQLNHANRMA AEALRNYRNT
1651 QAILKDTQLH LDDALRSQED LKEQLAMVER RANLLQAEIE ELRATLEQTE
1701 RSRKIAEQEL LDASERVQLL HTQNTSLINT KKKLETDISQ IQGEMEDIIQ
1751 EARNAEEKAK KAITDAAMMA EELKKEQDTS AHLERMKKNL EQTVKDLQHR
1801 LDEAEQLALK GGKKQIQKLE ARVRELEGEV ESEQKRNVEA VKGLRKHERK
1851 VKELTYQTEE DRKNILRLQD LVDKLQAKVK SYKRQAEEAE EQSNVNLSKF
1901 RRIQHELEEA EERADIAESQ VNKLRVKSRE VHTKIISEE
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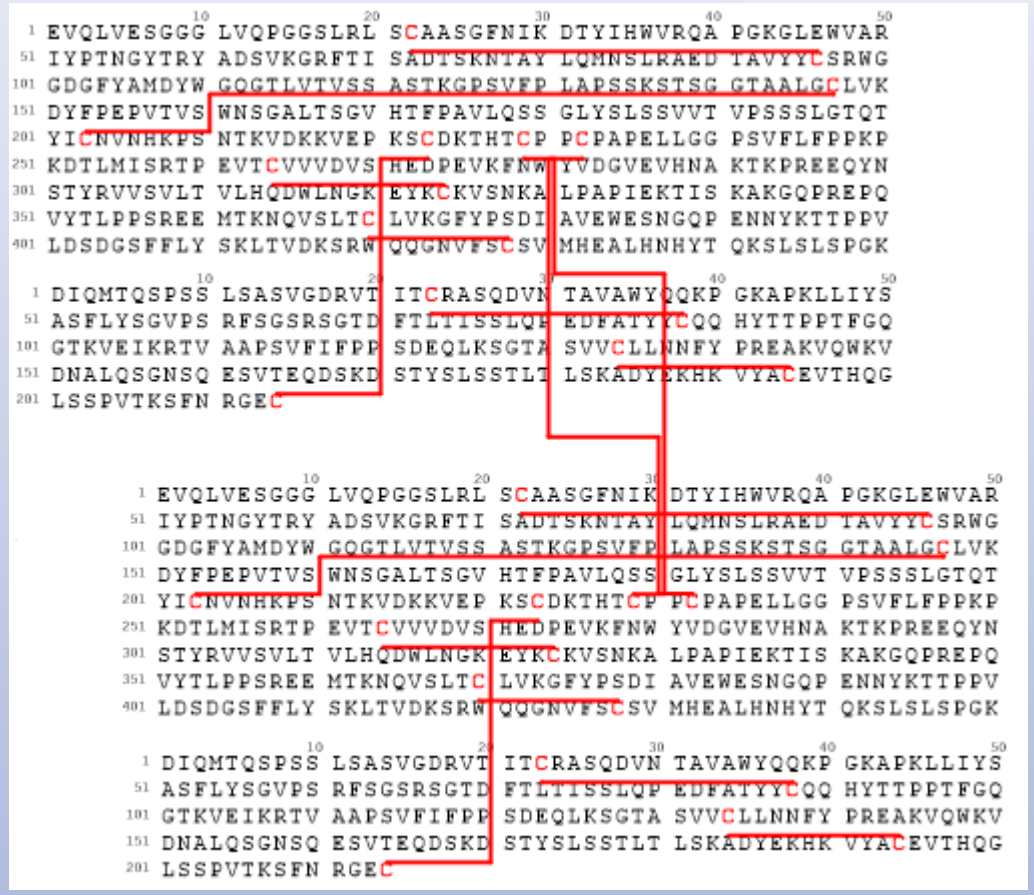
- Calculation time: ~94s
- Molecular Formula: $C_{9725}H_{15816}N_{2748}O_{3100}S_{72}$

Trastuzumab dimer

- InChIKey=VRBUFPXQWJVPLO-JNJMYDJTBA-N
- Single arbitrary stereocenter inverted
  - InChIKey=VRBUFPXQWJVPLO-RCEINSQCBA-N
- Calculation time: ~27s
- Molecular Formula: $C_{6460}H_{9972}N_{1724}O_{2014}S_{44}$



Summary

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- InChI v1.05 can generate InChI strings and keys from large structures
  - InChI strings are unwieldy
- All calculations were done using the winchi-1 application
  - Convenient to use but not the most efficient method for calculating InChI strings and keys
- Calculation time for Filgrastim related peptides and the synthetic erythropoietin were not perceptible using the winchi-1 program
- Processing time for large structures needs to be improved
- A large polynucleotide timed out but a polypeptide of similar size did not
- Myosin-1, presented as a linear peptide, took ~94s to process whereas Trastuzumab took ~27s
- Canonicalization may be an area of weakness
- Trastuzumab stereoisomers are differentiated
  - An arbitrary stereocenter in Trastuzumab was inverted
  - Processing time unchanged
  - Different InChI key was produced

Next Steps

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Trastuzumab emtansine: patent extract

The mertansine is conjugated to the trastuzumab through a maleimidocaproyl (MC) linker which bonds at the maleimide to the 4-thiovaleric acid terminus of the mertansine side chain and forms an amide bond between the carboxyl group of the linker and a lysine basic amine of the trastuzumab. Trastuzumab has 88 lysines (and 32 cysteines). As a result, trastuzumab emtansine is highly heterogeneous, containing dozens of different molecules containing from 0 to 8 mertansine units per trastuzumab, with an average mertansine/trastuzumab ratio of 3.4.

Suggestions

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- Remove intolerance of Sgroup data in molfiles
- Support HELM-2 and SCSR as input formats
- Investigate performance issues
  - Canonicalization
  - Timeouts
- Enhance InChI data model to support
  - Variable substitution
  - Variable loading
  - Hydrogen bonds
  - Organometallic bonding
- Remove arbitrary limits
  - In particular maximum atom limit

Question

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- Does InChI need to be a rigorous (valence-bond) representation of the structure?
- Is reproducible sufficient

Proposal

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- Extend format with extra layers
  - Base InChI correlates to unsubstituted substance
  - Variable substructure
  - Loading variation – 1 to n
  - Position of loading
  - Use new flag to identify that InChI contains variable substituents and variable loading
- InChI key may need third section to contain variability information